Jurnal Agro*Biogen* 7(1):37-46

Phylogenetic and Maturity Analyses of Sixty Soybean Genotypes Used for DNA Marker Development of Early Maturity Quantitative Trait Loci in Soybean

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Submitted: 6 Agustus 2010; Accepted: 28 Januari 2011

ABSTRAK

Analisis Filogenetik dan Kegenjahan 60 Genotipe Kedelai untuk Pengembangan Marka DNA Terkait Karakter Umur Genjah pada Kedelai. ***I Made Tasma, Dani Satyawan, Ahmad Warsun, Muhamad Yunus, dan Budi Santosa.*** Produktivitas kedelai Indonesia saat ini masih rendah dengan rataan nasional 1,3 t/ha. Salah satu cara meningkatkan produksi kedelai nasional dengan memanipulasi indeks panen menggunakan varietas super genjah. Pemuliaan kedelai super genjah dipercepat dengan menggunakan bantuan marka molekuler. Tujuan dari penelitian ini untuk memilih tetua-tetua dengan perbedaan kontras karakter umur genjah dan menunjukkan jarak genetik jauh. Tetua terpilih digunakan untuk membentuk populasi untuk pengembangan marka molekuler terkait umur genjah. Uji kegenjahan 60 genotipe kedelai dilakukan di dua lokasi, KP Cikeumeuh (Bogor) dan KP Pacet (Cianjur) menggunakan rancangan acak kelompok, tiga ulangan. DNA genomik 60 genotipe kedelai dianalisis menggunakan 18 marka SSR dan dendrogram kekerabatan antar aksesi dikonstruksi menggunakan *Unweighted Pair-Group Method Arithmatic* melalui program *Numerical Taxonomy and Multivariate System* versi 2.1-pc. Hasil penelitian menunjukkan bahwa distribusi normal 60 genotipe kedelai di kedua lokasi pada karakter waktu berbunga (32-48 hari), waktu berpolong (35-55 hari), waktu matang fisiologis (75-92 hari), dan waktu panen (78-99 hari). Diperoleh empat genotipe berumur genjah (umur masak tergenjah 75,3 hari dan warna *pubescent* coklat), tiga genotipe umur dalam (umur masak terdalam 89,7 hari dan warna *pubescent* abu-abu). Jumlah alel SSR total 237, rataan alel per lokus 12,6 (3-29), rataan nilai PIC 0,78 (0,55-0,89). Tingkat kesamaan genetik berkisar 74,8-95%. Pada kemiripan 77% membagi genotipe menjadi enam klaster (empat genotipe umur genjah ada pada klaster III dan IV dan tiga genotipe umur dalam ada pada klaster II). Berdasarkan analisis data umur, warna *pubescent*, dan analisis filogenetik terpilih tujuh tetua yang digunakan untuk pengembangan marka terkait umur genjah, yaitu empat tetua berumur genjah B1430, B2973, B3611, B4433 dan tiga tetua berumur dalam B1635, B1658, dan B3570. Dua belas populasi F_2 dibentuk menggunakan bantuan marka Satt300 dan Satt516. Dua di antara populasi tersebut dapat digunakan untuk pengembangan marka molekuler umur genjah.

Kata kunci: Marka molekuler, umur genjah, kekerabatan genetik, kedelai, QTL.

ABSTRACT

Phylogenetic and Maturity Analyses of Sixty Soybean Genotypes Used for DNA Marker Development of Early Maturity Quantitative Trait Loci in Soybean. ***I Made Tasma, Dani Satyawan, Ahmad Warsun, Muhamad Yunus, and Budi Santosa.*** The Indonesian soybean productivity is still very low with the national average of 1.3 t/ha. One means to improve national soybean productivity is by manipulating harvest index by cultivating very early maturing soybean cultivars. Development of early maturing soybean cultivars can be expedited by using marker-aided selection. The objective of this study was to select parental lines having contrasted maturity traits and selected parents must be genetically distance. The parents then were used to develop F_2 populations for detecting early maturity QTL in soybean. Maturity tests of 60 soybean genotypes were conducted at two locations, Cikeumeuh (Bogor) and Pacet (Cianjur) using a randomized block design with three replications. Genomic DNA of the 60 genotypes were analyzed using 18 SSR markers and genetic relationship was constructed using the Unweighted Pair-Group Method Arithmatic through Numerical Taxonomy and Multivariate System program version 2.1-pc. Results showed that the 60 genotypes demonstrated normal distribution in both locations for days to R1 (32-48d), days to R3 (35-55d), days to R7 (75-92d), and days to R8 (78-99d). Four early maturing genotypes and three late genotypes were obtained. Total SSR alleles observed were 237 with average allele per locus of 12.6 (3-29), and average PIC value of 0.78 (0.55-0.89). Genetic similarity among genotypes ranges from 74.8-95%. At similarity level 77% divided the genotypes into six clusters (the four selected early maturing genotypes located in clusters III and IV, while the three late genotypes located in cluster II). Based on maturity data, pubescent color, and phygenetic analysis seven parents were selected (four early maturing genotypes B1430, B2973, B3611, B4433 and three late genotypes B1635, B1658, and B3570). Twelve F_2 populations were developed with the aid of SSR markers Satt300 dan Satt516. Two of the populations will be used to develop DNA markers for earliness in soybean.

Keywords: Molecular marker, maturity, genetic distance, soybean, QTL.

INTRODUCTION

The 1990-2008 Indonesian soybean plantations showed significant decreases both in total areas and production with a total national area of 1.6 millions ha and total production of 1.87 million tons in 1992 to become only 0.592 million ha and 0.776 million tons in 2008. The national soybean consumption in 2008, however, reached 2.02 million tons. The deficit of 1.31 million tons (65%) was fullfiled through import (BPS, 2008).

The main constrains of the Indonesian soybean cultivation is the low productivity of only about 1.3 ton/ha, much lower than the main soybean produsers such as the USA, Brazil, and Argentine having national production up to 3.0 tons/ha (Marwoto *et al.,* 2005).

National soybean self-sufficiency can be achieved by increasing soybean productivity, plantation expansion outside the Java island and manipulation of harvest index (HI). Increasing HI means the use of very early maturity varieties so that more than two cultivations can be accomplished within a year. Developing a very early maturing soybean varieties with productivity similar to the ones available at present will be very crucial to achieve the national production goal.

Earliness level of a soybean genotype is determined by the quickness of the genotype to initiate flowers compared to the late maturing ones. The reproductive related traits are controlled by eight maturity genes named the *E* gene series (*E1-E8*) (Bonato and Vello, 1999; Cober *et al*., 2010). The genes were found to interact with day length (McBlain *et al*., 1987) that determines when the right time to flower. In addition a gene controlling the soybean growth habit (*Dt1*) also affect the time of flowering and maturity in soybean (Foley *et al*., 1986).

With the development of DNA markers, genetic maps of many plant species have been constructed including soybean (Tasma *et al*., 2001; Song *et al*., 2004). The genetic maps will be very useful in dissection of QTL for economically important characters including the ones related to early flowering and maturity. The availability of numerous SSR markers in the soybean map will facilitate detection of molecular markers linked to important traits in soybean. This is because SSR markers showed high polymorphisms across different artificial mapping populations. SSR polymorphism levels of SSR markers in soybean are generally more than 50% depending on the populations used (Tasma *et al*., 2001). In addition, the reference sequence of the soybean genome has been publicly available (http://www.phytozome.net/soybean.php) that will facilitate the gene-based marker discovery to support soybean breeding programs.

Development of DNA markers tightly linked to the trait controlling earliness will *expidate* breeding process as the selection can directly be done soon after seeds germinate. Selection of plants having super earliness character using marker-assisted selection (MAS) technology will save breeding resources including costs for experimental farms and man power that will expidate the very early maturing soybean cultivar development to support the national self-sufficiency program scheduled to be acheived in the year of 2015.

In this study molecular marker development will be conducted using F_2 populations. Parents will be selected by screening 60 soybean genotypes at two field conditions to select the very early and very late maturing soybean genotypes. The selected contrasted maturity phenotypes must demonstrate different pubescent colors and are genetically distance to facilitate high polymorphism. Pubescent color is the best marker for soybean maturity genes *E1* and *E7* located on chromosome C2, where major flowering time, maturity, and photoperiod sensitivity QTLs were previously mapped (Tasma *et al*., 2001; Yamanaka *et al*., 2000). Using different populations the QTLs explained up to 70% of phenotypic variances making this major QTL becomes of prominent interest for the purposes to develop early maturing soybean cultivars. This QTL is a targeted chromosomal region to be introgressed in the future breeding programs. F_2 populations are then developed by crossing the early and late maturing soybean genotypes having different pubescent colors and are used to phenotype early maturity-related traits. Polymorphic SSR markers between the two parents are screened and be used to genotype the F_2 populations. QTLs controlling the earliness are then identified by single factor analysis of variance using genotypic and phenotypic data and followed by interval mapping to identify chromosomal regions controlling the trait using a MapMaker/QTL computer program (Lincoln and Lander, 1993).

The objectives of the present study were to obtain soybean genotypes having contrasted early-maturity related traits with different pubescent colors and demonstrated enough genetic distances. The selected parents will be used to develop F_2 populations for developing molecular markers for earliness in soybean.

MATERIALS AND METHODS

Maturity Analyses of 30 Early and 30 Late Maturing Soybean Genotypes at Two Field Locations

Thirty early and 30 late maturing genotypes of the ICABIOGRAD soybean collections (Table 1) were tested at two West Java field conditions. The experiments were conducted at the Cikemeuh Experimental Station, Bogor (250 m above sea level, asl) and at the Pacet Experimental Station, Cianjur (1.000 m asl). The primary objective of this study was to observe the maturity and pubescent color performance of the tested genotypes at the lowland (Bogor) condition as most soybean cultivation in Indonesia is at lowland. As a comparison another study was conducted at higher altitude (Cianjur). The time of flowering and maturity will be delayed at a certain degree at the higher altitude compared to that observed at the lower altitude. We then used the Bogor data for further analyses.

The extreme earliness and lateness genotypes having different pubescent colors were selected and be used for DNA marker development for early maturity trait. As previously described, pubescent color is the best marker for maturity genes *E1* and *E7*, located on chromosome C2. The experiments were conducted using a randomized block design with three replications. Individual soybean genotypes were planted using spacing of 0.5 m (between lanes) x 0.2 m (within lanes) planted with two seeds per hill. Plants were fertilized with dung manure (2 tons/ha), 50 kg urea, 100 kg SP-36, and 150 KCl per hectare. To control insect attacks, plants were sprayed weekly with insecticide Decis. Parameters observed included days to R1 (the day after emergence when 50% of the plants in a plot have an open flower at one of the top nodes with a fully expanded leaf), days to R3 (the number of days after emergence when 50% of plants in a plot had presented the first 5 mm pod at one of the top four nodes with fully expanded leaf), days to R7 (the number of days after emergence when 50% of pods within a plot had mature pod color), and days to R8 (the number of days after emergence when 100% of pods within a plot had mature pod color) (Fehr and Caviness, 1977; Fehr, 1987). Data were analyzed using analysis of varance (ANOVA) (Steel and Torrie, 1980; SAS, 1990).

The earliest genotypes, should show days to R7 as close as 70 days after planting the seeds as our original objective was to obtain the ones with days to R7 approached 70 days. The extreme late maturity was determined where the genotypes showed days to R7 approached 20 days latter than that demonstrated by the early maturing genotypes. This is because of the effects of the dominant allele of the maturity gene *E1*. The dominant allele *E1*delayed days to R7 14-20 days compared to the early allele *e1*. The two contrasted genotypes, however, must show different pubescent colors which are light brown to brownish orange (tawney) and grey.

Phylogenetic Analysis of 30 Early and 30 Late Maturing Soybean Genotypes Using Micro Sattelite Markers

Studies were conducted at the ICABIOGRAD Biology Molecular Laboratory, Bogor, West Java, from May to December 2009. The primary objective of this study was to understand the genetic relationship among the 60 soybean genotypes tested. The hypothesis was that among the phenotypic divergent genotypes observed based on the phenotypic analyses some are geneticall-ly divergent based on SSR marker assays and should be useful for the purposes of F_2 population development. Genetic materials used were the same as those used in the phenotypic analysis study (Table 1).

Genomic DNA Isolation

Sixty genotypes were planted in pots grown in a glass house. Young leaf of a two-week old plant was used for genomic DNA isolation. Similar amount of leaves (originated from 10 individual plants per genotype) was harvested. DNA was isolated by following the method of Keim *et al*. (1988) using CTAB extraction buffer. The quality of DNA was tested using the agarose gel electrophoresis standard method and the relative DNA quantity of each DNA sample was measured using the standard Lambda DNA (Sambrook *et al*., 1989).

PCR, Electrophoresis, and Data Scoring

SSR amplification was conducted using the method of Akkaya *et al*. (1995) by using the PCR Core System of Promega (Promega, Wisconsin, USA). A PCR reaction of 20 μl total volume was conducted in an MJ Research 96-well PCR machine (MJ Research, New Jersey, USA) for 45 cycles. A total of 18 SSR markers (Table 2) was assayed through the PCR study. PCR products for each SSR primer pairs were then separated in a 5% polyacrylamide gel electrophoresis using 0.5 x TBE buffer using a Vertical SesquiGen GT 38 x 30 electrophoresis aparatus from Bio Rad (Bio Rad, California, USA). Gels were then stained using the silver staining method by following the standard protocol (Sambrook *et al*., 1989). The separated DNA was scanned, scored, and analyzed.

Table 1. Genotypic code and genetic background of 30 early and 30 late maturing soybean genotypes used in this study.

No.	Genotypic code	Genetic background	Previously identified as[a]
1.	B0887	Genjah Slawi	E
2.	B1306	Black Mancuria	E
3.	B1430	Semmes	E
4.	B2973	G.6475	E
5.	B2981	G.7470	E
6.	B3293	Genjah Hitam	E
7.	B3414	Green soybean	E
8.	B3417	1004/1343-68-9	E
9.	B3562	GM.915si	E
10.	B3611	A landrace from Kediri	E
11.	B3626	A landrace from Sampang	E
12.	B3628	AVRDC-G2160 mb	E
13.	B3633	G.2120 mb	E
14.	B3753	M.2969	E
15.	B3897	Plant introduction (PI) from Koyu, S. Korea	E
16.	B4176	PI24807 x PI 867736	E
17.	B4182	C.73-01-1217-c-pop	E
18.	B4229	Langkat soybean	E
19.	B4334	M.3200	E
20.	B4334	M.3200	E
21.	B4395	GM.796si	E
22.	B4439	SS2-2	E
23.	B4125	PWR-6	E
24.	B4433	A landrace from Grobogan	E
25.	B4440	Lokal Jenggot	E
26.	B4441	A landrace from Pringgorita W. Nusa Tenggara	E
27.	B4432	Gepak Ijo	E
28.	B4431	Gepak Kuning	E
29.	B4429	Detam-1	E
30.	B3492	Tambora	E
31.	B1312	PI/HS/2	L
32.	B1593B	A landrace from Bali	L
33.	B1635	Presi soybean	L
34.	B1658	A landrace from Sopeng, S. Sulawesi	L
35.	B1958	PI Columbus	L
36.	B3185	Lokal Kerok	L
37.	B3193	Lokal Godek	L
38.	B3367	Lokal Godek	L
39.	B3391	Lokal Kebumen	L
40.	B3442	Lumut (aluminum-toxicity sensitive variety check)	L
41.	B3498	Lokal Kretek Balap	L
42.	B3517	Breeding line 7702(ckIV-6/2299)	L
43.	B3558	Breeding line 1312/317	L
44.	B3570	Breeding line 11682/1248	L
45.	B3625	M.3739	L
46.	B3648	A landrace from Sukoharjo	L
47.	B3686	A landrace from Tabanan-Bali	L
48.	B3778	M.3000	L
49.	B3787	M.3011	L
50.	B3799	M.3027	L
51.	B3802	M.3030	L
52.	B3841	M.2566	L
53.	B3898	Breeding line LB-80	L
54.	B3907	Breeding line 4/8/10/4/0	L
55.	B3918	GM.216 si	L
56.	B4304	Breeding line IB-2/2	L
57.	B4309	BPTP Krp-3 (a landrace)	L
58.	B4311	M.3208	L
59.	B4214	Breeding line 4/8/2/6/0	L
60.	B4382	GM.2841 si	L

[a]E = early pod maturity, L = late pod maturity based on previous studies.

Table 2. The characteristics of the SSR markers used in the phylogenetic study using 30 early and 30 late maturing soybean genotypes.

Marka SSR	Chromosome/*Linkage group*	Gene bank accession number	Repeatation types	The segregation ratio in the reference F_2 population *G. max* X *G. soja*[a]
Sat_022	D2	BH126254	(AT)27	13A : 28H : 11B
Sat_043	K	CC453677	(AT)21	12A : 32H : 15B
Sat_069	D1b	CC453679	(AT)29	17A : 30H : 14B
Satt012	G	BH146213	(ATT)19	14A : 34H : 12B
Satt070	B2	BH126318	(ATT)24	16A : 29H : 12B
Satt100	C2	BH126330	(ATT)33	11A : 28H : 14B
Satt300	A1	BH126491	(ATT)19	13A : 33H : 13B
Satt409	A2	BH126589	(ATT)27	13A : 32H : 13B
Satt230	E	BH126431	(ATT)15	14A : 34H : 11B
Satt131	G	BH126345	(ATT)13	18A : 26H : 14B
Satt222	H	BH126425	(ATT)20	17A : 28H : 14B
Satt516	F	BH126686	(ATT)19	11A : 27H : 17B
Satt446	L	BH126621	(ATT) 21	11A : 33H : 12B
Satt285	J	CC453960	(ATT) 19	12A : 32H : 14B
Satt_289	C2	BH126482	(ATT)16	16A : 29H : 15B
Satt_489	C2	BH126660	(ATT)23(GTT)8(ATT)8	14A : 32H : 14B
Sat_251	C2	CC453787	(AT)19	18A : 28H : 14B
Sat_336	C2	CC453862	(AT)19	13A : 30H : 17B

[a]The segregation ratio of the primer pairs used was determined in an F_2 population of an inter-specific cross between *G. max* breeding line A7861 and *G. soja*. The null hypothesis of the test was that markers segregated in a 1 : 2 : 1 ratio of allelles originated from *G. max*, from both parents (*G. max* and *G. soja*) and *G. soja*, respectively (Cregan *et al*., 1999, Song *et al*., 2004). The size of the *G. max* x *G. soja* population was 60 individual F_2. All the selected primers followed the segregation ratio 1 : 2 : 1 of a single locus segregation ratio.

Data Analysis

Each DNA band in a gel represents DNA fragment of a plant genotype and was given a value of 1 when the band exists and 0 when no band appears. Genetic similarity was calculated using the method of Rohlf (2000). The polymorphism information content (PIC) values for each SSR marker was calculated using the formula of Smith *et al*. (1997) as previously reported (Tasma and Warsun, 2009). A dendrogram then was constructed using the Unweighted Pair Group Method Arithmetic (UPGMA) method through the Numerical Taxonomy and Multivariate System (NTSYS) program version 2.1-pc (Rohlf, 2000).

Development of F_2 Population Using the Aid of SSR Markers

Experiments were conducted at the Cikeumeuh Experimental Station, Bogor from August to December 2009. The selected early and late maturing soybean plants showing different pubescent colors (Table 3) were grown in pots containing 10 kg soils each enriched with dung manures. Three plants were grown per pot. Seeds were sown in such away therefore the two parents to be crossed flower in approximately the same time. About 5-8 crossings were done in each combination, tagged with violet string, labeled, and, dated. Twelve crossing combinations were done by crossing four early and three late maturing soybean genotypes. The putative F_1 seeds from each cross were harvested separately.

The putative F_1 plants were tested their heterozygosity using SSR markers. Putative F_1 seeds (8 seeds per cross) were sown in pots, one seed per pot. DNA was isolated from leaf of a 7-day old F_1 plant using a method of Keim *et al*. (1988). DNA quality and quantity were determined using the method of Sambrook *et al*. (1989). The heterozygosity of the F_1 was determined using SSR markers Satt300 or Satt516 because the two SSR markers showed polymorphism in the parent-pairs used in this study. The PCR, electrophoresis, and data scoring of the two SSR markers are the same as that done in the phylogenetic analysis studies of the 60 genotypes. True F_1 must be heterozygote (i.e., showing DNA bands originated from both parents). F_2 seeds of the selected true F_1 plants were harvested separately and be used for further experiments.

RESULTS AND DISCUSSION

Maturity Performance of 30 Early and 30 Late Maturing Soybean Genotypes at Two Field Locations

F test of the analysis of variance (ANOVA) showed that genotypes tested in this study demonstrate significant differences on days to R1, days to R3, days to R7, and days to R8 in both study locations (Table 4). The results indicated that the genotypes tested are well representated by variable alleles related to time of flowering and maturity. The genotypes tested showed similar delay in time of flowering and maturity at higher altitude.

Table 3. The selected four early and three late maturing soybean genotypes having different pubescent colors based on phenotypic studies at the Cikeumeuh Experimental Station, Bogor (May to September 2009).

Genotypes	Days to				Pubescent color[a]
	R1	R3	R7	R8	
Early maturing genotypes					
B1430	36.7	44.3	75.3	78.7	Tawney
B2973	36.7	43.7	75.3	78.3	Tawney
B3611	35.3	42.7	76.7	78.3	Tawney
B4433	32.7	35.3	77.3	79.7	Tawney
Late maturing genotypes					
B1635	47.3	53.7	88.7	94.7	Grey
B1658	46.7	50.7	89.7	95.7	Grey
B3570	48.0	54.7	89.7	96.3	Grey

[a]Pubescent color is controlled by a single locus, *T*, mapped in the middle of chromosome C2. The *T* locus is a well-known marker for maturity genes *E1* and *E7*.

Table 4. Statistical analysis results on the effect of soybean genotypes on days to R1, R3, R7, and R8 observed at the Cikeumeuh Experimental Station, Bogor (May-September 2009) and Pacet Experimetal Station, Cianjur (July-November 2009).

Traits	Mean square	F-value	Pr>F
Cikeumeuh Experimental Station, Bogor (May-September 2009)			
Days to R1	44.833570	17.09	<0.0001
Days to R3	42.324906	8.67	<0.0001
Days to R7	34.493119	9.98	<0.0001
Days to R8	490.50092	6.93	<00001
Pacet Experimental Station, Cianjur (July-November 2009)			
Days to R1	49.749435	16.58	<0.0001
Days to R3	45.128437	11.07	<0.0001
Days to R7	158.523070	15.99	<0.0001
Days to R8	123.800753	10.45	<0.0001

Histograms on the relationship between genotypic frequency and the four reproductive traits (days to R1, R3, R7, and R8) demonstrated that the traits are normally distributed (Figure 1). The ditributions clearly indicated that the four traits are controlled by more than two loci. A similar distribution was shown by other previous studies observing the four characters using different populations and locations (Yamanaka *et al*., 2000; Tasma *et al*., 2001). The results are in agreement with the previous studies showing that the reproductive-related traits in soybean are controlled by quantative traits (Tasma *et al*., 2001; Yamanaka *et al*., 2000; Lee *et al*., 1996). This study also supports the results derived from the model plant *Arabidopsis thaliana* indicating that the transition from vegetative phase to flowering phase is complex and is controlled by many genes and by several genetic pathways (Levy and Dean, 1998; Koernneef *et al*., 1998). The phenotypic data further indicated that the genotypes tested represent well the allelic variations among the genotypes tested suggesting an excellent selection of the plant genetic materials used in this study.

Among the 60 genotypes tested, four genotypes were identified to demonstare extreme early time of flowering and maturity and three genotypes demonstrated extreme late time of flowering and maturity. The four genotypes showing early flowering and maturity showed a tawney pubescent color, while the three late flowering and maturity genotypes showed a grey pubescent color (Table 3).

Phylogenetic Analysis of 30 Early and 30 Late Maturing Soybean Genotypes Based on SSR Marker Assays

The genetic relationship study of the 30 early and 30 late maturing soybean genotypes (Table 1) by using 18 SSR markers (Table 2) was conducted at the Molecular Biology laboratory of ICABIOGRAD. The total SSR alleles observed was 237. The average allele per locus was 12.6 (ranges from 3 to 29) (Table 5). The average polymorphism information content (PIC) value of the 19 SSR markers tested in the 60 soybean genotypes was 0.78 (ranges from 0.55 to 0.89) (Table 5). The allelic diversity and polymorphism level observed in this study is much higher than those

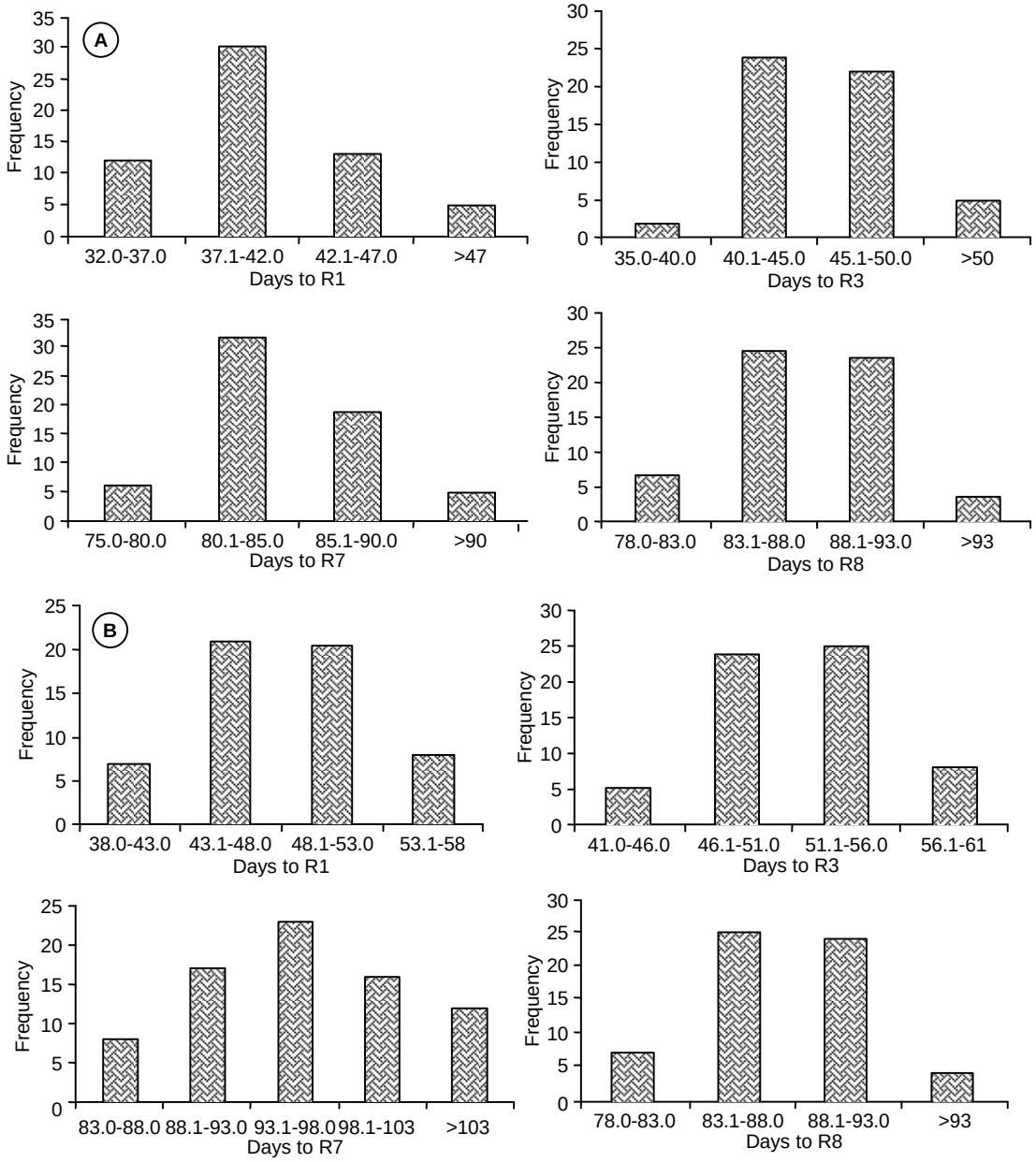

Figure 1. Histograms of days to R1, days to R3, days to R7, and days to R8 derived from data of the 60 soybean genotypes tested at two field locations. A = Cikeumeuh Experimental Station, Bogor (250 m asl), B = Pacet Experimental Station, Cianjur (1000 asl).

previously observed, generally near 50% or above (Diwan *et al*., 1997; Tasma and Warsun, 2009). This study showed PIC values of 55-89%. This finding indicated that if we selected divergent genotypes with a larger number to be genotyped the PIC values become higher than that using a lower number of genotypes as clearly shown by this study.

Cluster analysis using the highest genetic similarity level of 77% divided the accessions into six groups (Figure 2, Table 6). The selected four early maturing soybean genotypes (Table 3) are located within clusters III and IV, while the three late maturing soybean genotypes (Table 3) are located within cluster II (Figure 2, Table 6). Based on data analyses of time of flowering and maturity, pubescent color, phylogenetic analysis, SSR marker profiles, the four early (B1430, B2973, B3611, and B4433, Table 3) and the three late maturing soybean genotypes (B1635, B1658, and B3570, Table 4), were selected and used as parents for the F_2 population development.

Table 5. Profiles of 18 SSR markers used to assay 30 early and 30 late maturing oybean genotypes.

SSR marker	Number of alleles detected	Polymorphism level (PIC values)
Sat_022	18	0.85
Sat_043	29	0.86
Sat_069	8	0.72
Satt012	17	0.88
Satt070	18	0.88
Satt100	8	0.70
Satt300	9	0.84
Satt409	17	0.89
Satt230	5	0.71
Satt131	18	0.85
Satt222	13	0.87
Satt516	11	0.80
Satt043	29	0.86
Satt289	3	0.73
Satt489	5	0.55
Sat_251	6	0.65
Sat_336	4	0.60
Satt_312	9	0.80
Average	12.6 (3-29)	0.78 (0.55-0.89)

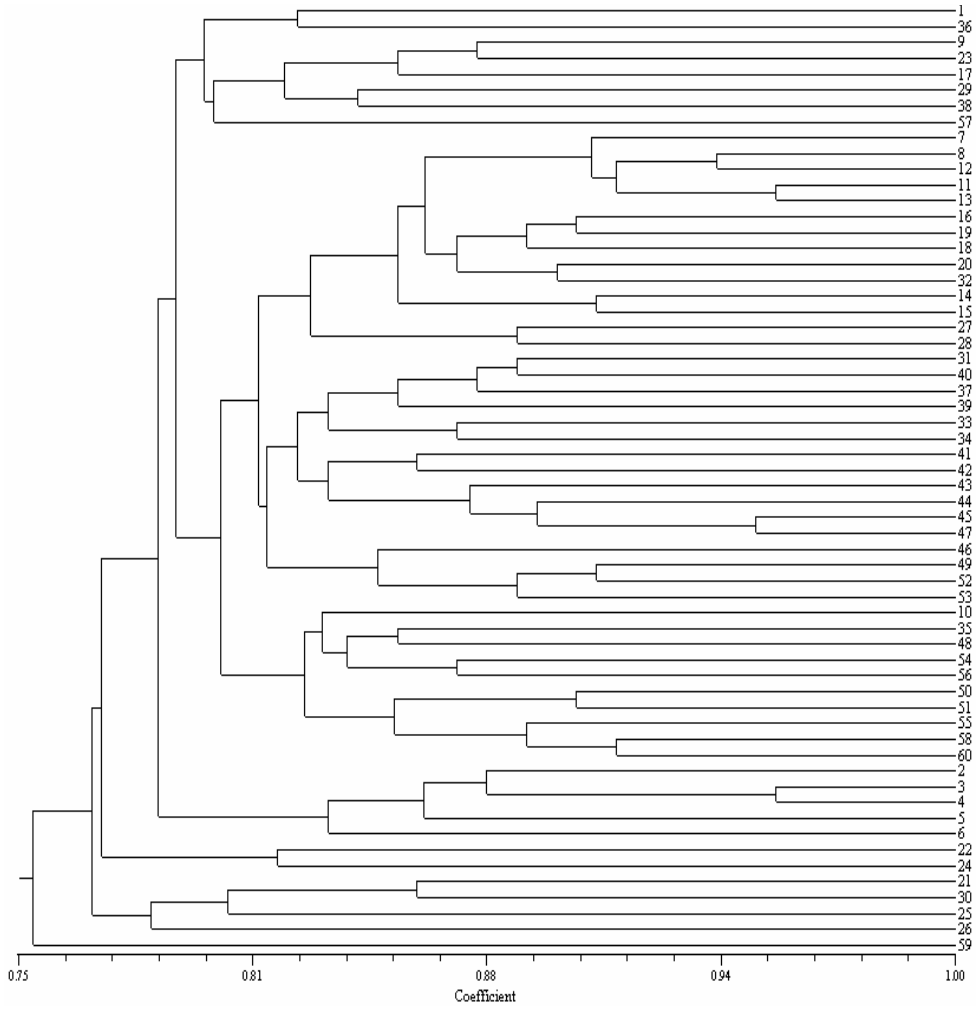

Figure 2. Dendrogram of 30 early and 30 late maturing soybean genotypes assayed with 18 SSR markers. Numbers in the right column of the dendrogram corresponded to the genotypes shown in Table 1.

Table 6. Clustering of the 60 soybean genotypes at the similarity level of 77% based on cluster analysis results using 18 SSR markers.

Cluster	Soybean genotypes included in each cluster	Number of accessions included in each cluster
I	B0887, B3185, B3562, B4125, B4182, B4429, B3367, B4309	8
II	B3414, B3417, B3628, B3626, B3633, B4176, B4229, B4334, B4381, B1593B, B3753, B3897, B4432, B4431, B1312, B3442, B3193, B3391, B1635, B1658, B3498, B3517, B3558, B3570, B3625, B3686, B3648, B3787, B3841, B3898, B3611, B1958, B3778, B3907, B4304, B3799, B3802, B3918, B4311, B4382	40
III	B1306, B1430, B2973, B2981, B3293	5
IV	B4439, B4433	2
V	B4395, B3492, B4440, B4441	4
VI	B4214	1

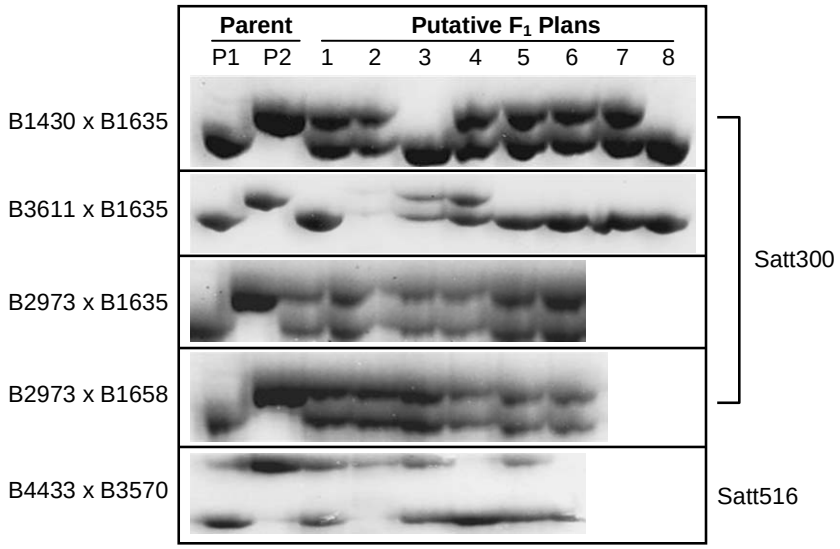

Figure 3. Confirmation of F_1 plants from crosses of early maturing and late maturing parents using SSR markers Satt100 and Satt516. P_1, female parent, P_2, male parent. 1-8, the putative F_1 plants.

F_2 Populations Development through the Assisstance of SSR Markers

Twelve crossing combinations of the four early and three late flowering and maturity soybean genotypes were done. The putative F_1 plants were identified their banding patterns using SSR markers Satt300 and Satt516. Satt 300 and Satt516 were used because they showed high polymorphism between pairs of parents used in this study. The selected F_1 plants are the ones showing heterozygote DNA bands (i.e., the plants having DNA bands originated from both parents) (Figure 3). For example in population B1430 x B1635, the putative plants number 1, 2, 4, 5, 6, and 7 were heterozygotes and therefore, are true F_1, while plants number 3 and 8 were not, and therefore, they were not F_1 but having SSR allele from the female parent. Similarly for population B3611 x B1635 only plants number 3 and 4 were heterozygotes and hence real F_1, other putative F_1 plants in this population did not show heterozygote bands, and therefore, they were not F_1. Heterosigosity analyses of allpopulations shown in Figure 3 showed that 37.5 to 100% of the plants tested were true F_1. This result is similar to that reported in the previous study (Tasma *et al*., 2008). For QTL mapping purposes only a single F_1 plant is used for F_2 population development. A single F_1 plant was then selected from each cross and the selected F_1 plants were self-pollinated to obtain F_2 seeds.

CONCLUSION

Based on data of time of flowering and maturity, pubescent color, and phylogenetic tree of the 30 early and 30 late maturing soybean genotypes, four early maturing (B1430, B2973, B3611, and B4433) and three late maturing genotypes were selected as parents to be used for DNA marker development of early maturity QTL in soybean. The four early maturing soybean genotypes are located in clusters III and IV of the phylogenetic tree and demonstrated tawney pubescent color. The three late maturing soybean genotypes, on the other hand, are located in cluster II and demonstrated a grey pubescent color. Twelve F_2 popu-

lations were developed and two of the populations will be used for molecular marker development of early maturity QTL in soybean.

ACKNOWLEDGEMENTS

This study was funded by the 2009 Sinta Project, Directorate General for Higher Education of the Ministry of Education. The authors thank Mr. Fajar Suryawan, Mrs. Ratna Utari, Mrs. Ma'sumah, ICABIOGRAD Cikeumeuh and Pacet Experimental Station staffs and technicians for their involvement in conducting this project.

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