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Development and Characterization of F_2 Population for Molecular Mapping of Aluminum-Toxicity Tolerant QTL in Soybean

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ABSTRAK

Pembentukan dan Karakterisasi Populasi F_2 untuk Pemetaan QTL Karakter Ketahanan Tanaman Kedelai terhadap Keracunan Aluminium. ***I Made Tasma, Ahmad Warsun, dan Asadi*****.** Keracunan aluminium merupakan salah satu kendala utama dalam budidaya kedelai pada lahan masam. Pembentukan populasi F_2 merupakan langkah awal yang menentukan keberhasilan program pemuliaan tanaman. Tujuan penelitian ini untuk membentuk dan mengkarakterisasi populasi F_2 hasil persilangan tetua toleran dan tetua peka keracunan Al. Pembentukan populasi dilakukan menggunakan bantuan marka SSR. Dengan marka SSR populasi dapat dibentuk dengan cepat, akurat, dan efisien. Skrining genotipa kedelai pada tanah masam kahat hara menghasilkan dua genotipa toleran dan dua peka. Empat persilangan tunggal dibuat untuk mendapatkan benih F_1. Tanaman F_1 dan F_2 diidentifikasi menggunakam marka SSR Satt_070. Dua populasi (B3462 X B3293 dan B3462 X B3442) dipilih berdasarkan superiotas fenotipa pada lahan masam dan karakteristik molekuler pasangan tetua. Karakterisasi kedua populasi di lapang menunjukkan *transgresiveness* luas untuk karakter reproduksi seperti jumlah polong dan berat 100 biji. Ini mengindikasikan bahwa karakter penting lain selain karakter ketahanan terhadap keracunan Al potensial untuk dipetakan dari populasi ini. Metoda pembentukan populasi ini akan sangat bermanfaat bagi pemulia tanaman khususnya pemulia kedelai untuk meningkatkan efisiensi program pemuliaan ketahanan terhadap keracunan Al.

Kata kunci: Populasi, pemetaan molekuler, keracunan aluminium, kedelai.

INTRODUCTION

The Indonesian national soybean production is much lower than its annual consumption and, therefore, import is an option to fill in the production gap. As human population growth increases the value of the annual import also increases. For example in 1990 the total import was 0.541 million tons from the total consumption of 2.028 million tons. In 2004 the total import becomes 1.307 million tons from a total national consumption of 2.015 million tons (Marwoto *et al*. 2005). The government, however, has launched a mission to achieve soybean national self sufficiency by the year of 2015.

One of the strategies to increase national soybean production is by the use of large hectarage of acid soils available for soybean cultivation. The Indonesian acid soils are predicted to cover area of more than 101,519 million hectares distributed across the country and mainly located in the islands of Sumatera and Kalimantan (Notohadiprawiro 1983). The main constrain of agricultural activities in acid soils, however, is that such a soil exhibits aluminum toxicity, less availability of nutrients N, P, K, Ca, Mg, and Mo and less or no activity of the soil microbes (Mariska *et al*. 2004). At soil pH below 5, aluminum toxicity is the most serious problem faced by farmers in acid soils. At this situation soil is dominated by Al^{3+} solutes that are very toxic to plants (Kochian 1995). Al toxicity causes inhibition of root growth by stopping the root cell division, root penetration ability decreases and plant productivity drastically decreases (Mossor-Pietrazewska 2001, Delhaize *et al*. 2004, Hoekenga *et al*. 2006).

The productivity recovery of this marginal soil by increasing soil pH through liming of top soil and by amelioration with gypsum of sub soil and the application of macro nutrients N, P, K, and Mg has been successful to make the Savanah acid soils of Brazil to become healthy and productive (Spehar 1995). However, this method is valued to be too expensive to be applied in Indonesian and such an activity must be supported by a strong governmental policy to be able to exploit the huge hectarage Indonesian acid soil to become highly productive soils and suitable for soybean cultivation.

The use of Al-toxicity tolerant soybean varieties is a very attractive alternative to be considered. By this way the need to apply lime and fertilizer will be significantly decreased than the use of common soybean varieties. Variety development tolerance to Al toxicity could be expedited by using a better screening methodology. Screening methods used currently are varied among soybean breeders (Campbell and Carter 1990, Foy *et al*. 1993). The conventional screening methodologies through observing phenotype is less reliable

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and therefore less accurate as phenotypes are strongly affected by the environment (Bianchi-Hall *et al*. 1998, Ermolayev *et al*. 2003). A biotechnological method by using DNA marker will solve the problem. By using DNA markers linked to the trait cultivar development will be faster as selection of plants having the trait can be done at early stages of the plant growth. With DNA marker breeders will be able to select the plants having the trait without growing them in the environments where the trait is expressed.

Tolerance of soybean germplasm to Al-toxicity has been identified (Arsyad *et al*. 2002, Asadi, unpublished results). A series of populations by crossing the Al-toxicity tolerance and Al-toxicity sensitive genotypes can be done. Such a population will be used to dissect the Al-toxicity tolerance characters of soybean. We will apply SSR markers to tag the trait in various environmenttal conditions. The markers then will be used in a marker-aided selection of the traits in an Al-toxicity tolerance soybean breeding program.

The use of SSR marker in determining the heterozygote plants in early generation of plants growth expedites the determination of the F_1 plants as the heterozygote genotype can be verified at early generation (even at the seedling stage) of the plant growth. Using the conventional marker (e.g., seed coat color) at least two full generations will be needed to make sure that your selected F_1 is true segregating in the F_2. Using SSR marker we confidently select and grow the correct F_1 and eliminating the ones that are not F_1. This will save costs for growing the undesired plants the way that must be done in the common, conventional method of the population development. The reliability of the heterozygote plants selected using SSR markers is much higher than the one using conventional phenotypic marker due to the strong effect of the environmental factors of such a classical phenotypic marker.

In cultivar development by hybridization, the formation of a segregating F_2 population is the first step of a plant breeding program (Fehr 1987). The F_2 population is a genetic base from where all more developed lines are derived. Having an excellent F_2 population, therefore, is a requirement for the success of a cultivar development program.

The objectives of this study were to develop and characterize F_2 populations of crosses between the Al-toxicity tolerance and Al-toxicity sensitive parents. We reported here how the F_2 populations were precisely and efficiently developed by using the power of the co-dominance microsattelite DNA marker.

MATERIALS AND METHODS

Glass-house Study of 24 Soybean Genotypes using Poor-Nutrient Acid Soils

Study was conducted at the BB-Biogen glass house, Bogor, from March to June 2006 using a randomized block design with five replications. Treatments were 23 soybean genotypes as shown in Table 1. Acid soils were taken from Cipanas, Jasinga, West Java. Before used, soils were air-dried, sieved, and thoroughly mixed. The experimental unit was a pot (polybag) of size 30 x 20 cm containing 3 kg acid soil. The soil pH was 4.1 with an exchangeable Al of 15.52 me/100 g soil, and Al saturation of 75.82%.

Six seeds were sown per pot then thinned to five plants per pot two weeks after sowing. Plants were watered with raining water collected from rains fell on the BB-Biogen glass house. No fertilizers were applied. Plant growth was depending on the nutrients available from the Jasinga acid soils only. The objective of this experiment was to obtain the soybean genotypes that are able to tolerate the toxicity the most (most tolerance) and genotypes be able to tolerate toxicity the least (most sensitive) to Al toxicity in the stress of nutrients. Parameters observed were plant height (50 days after sowing, DAS), aluminum-toxicity chlorotic scores (30 DAS), pod numbers per plant (60 DAS), and seed numbers per plant during harvest. The value of aluminum-toxicity chlorotic scores were determined using the criteria shown in Table 2.

Development of F_2 Population with the Aid of SSR Marker

Studies were initiated in a field located near the Bogor BB-Biogen glass house. A total of four cross combinations was developed using three tolerant (B3462, B4378, and B3851) as female and two sensitive (B3293 and B3442) as male parents. The tolerant and sensitive plants 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, labelled, and dated. The putative F_1 seeds from each cross were harvested separately.

The putative F_1 plants were tested their heterozygosity using SSR markers. True F_1 must be heterozygote (i.e., showing DNA bands originated from both parents). Putative F_1 seeds (10 seeds per cross) were sown in pots, one seed per pot. DNA was isolated from leaf of 7-day old F_1 plants using a method of Keim *et al*. (1988). DNA was then diluted in TE buffer and their

Table 1. Genotypes and genetic background of 23 soybean genotypes used in the glass house Al-toxicity tolerant screening study.

Genotype	Genetic background	Previously identified as*
B4408	Wilis x No. 3898	T
B4378	Kerinci x No. 3911	T
B4375	Dempo x No. 3623	T
B4372	Dempo x Wilis	T
B3866	Dempo x Wilis	T
B4374	B3034 x Landrace Lampung	T
B4409	Somaclonal variation derived from Sindoro	T
B4410	Somaclonal variation derived from Sindoro	T
B4411	Somaclonal variation derived from Sindoro	T
B4412	Somaclonal variation derived from Sindoro	T
B4403	A landrace from Sukamandi	T
B3462	Plant Introduction from IRRI, the Philippines	T
B4376	B3034 x A landrace from Lampung	T
B3851	No. 1592 x Wilis	T
B4377	Dempo x No. 3577	T
B3442	A landrace from Tegal Gondo (Central Java)	S
B4368	Derived from Pedigree No. 27	S
B3293	A landrace from Kediri	S
B3531	Malang-1	S
B3614	A landrace from Pasuruan	S
B1958	Plant Introduction-USDA/USA	S
B3776	A landrace from Malang	S
B4311	Malang-2	S
B3898	A landrace from Bali	S

T = tolerance, S = sensitive to aluminum toxicity based on previous studies.

Source: Arsyad *et al*. (2002), Mariska *et al*. (2004), Somantri *et al*. (2004).

Table 2. The Al-toxicity chlorotic scores, tolerance levels, and chlorotic symptoms shown by soybean plants 30 days after showing (DAS).

Score	Tolerance level	Symptoms on soybean plant
0	Very tolerant	Green leaf, no Al-chlorotic symptom observed
1	Tolerant	Al-toxicity symptom appears around leaf tip, plant growth normal
2	Almost tolerant	Clearer chlorotic symptom, plant growth medium
3	Almost sensitive	Clear chlorotic symptom, plant growth inhibited (bad)
4	Sensitive	Chlorotic symptom very clear, plant growth very bad

Source: Asadi (unpublished result).

quality was tested using a standard 0.8% agarose gel electrophoresis. DNA quantity was determined using the undigested Lambda DNA standard, diluted with ddH_2O to make a concentration of about 30 ng/µL. The heterozygosity of the F_1 was determined using SSR marker Satt_070. Characteristics of the SSR marker Satt_070 are shown in Tables 3 and 4. PCR reaction was conducted using a previously published work (Akkaya *et al*. 1995) by using a PCR core system from Promega (Promega, Madison, Wiskonsin, AS). PCR reaction of volume 20 µl each containing 25 ng DNA, 1 x bufer PCR (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin), 100 µM dNTP mix, 1 µL each of 0.25 µM primer forward dan reverse and one unit of Taq DNA-polymerase was conducted using a termo-cycler model MJ Research 96-well (MJ Research, New Jersey, AS) for 45 cycles. Amplification products were then separated using 5% polyacrylamide gel electro-phoresis in 0.5 x TBE bufer using a Vertical SesquiGen GT 38 x 30 of BioRad (BioRad, Hercules, California, AS). Gels then were stained using a silver staining method by following the protocol (Sambrook *et al*. 1989). The separated DNA were then scanned using a Gel Doc gel scanning from BioRad (BioRad, Hercules, California, AS). The confirmed F_1 plants were grown to obtain F_2 seeds and the non F_1 plants were discarded.

Characterization and Confirmation of the F_2 Population

The putative F_2 plants were grown at the BB-Biogen's Pacet Experimental Station, Cianjur, West Java from September to December 2006. F_2 seeds derived from a single F_1 plant were grown in pots. Each pot contained 10 kg soils enriched with 0.5 kg dung manure. Only a single F_2 seed was planted per pot. Pest and disease managements were conducted as needed using the standard soybean cultural practices. Together with the parents, DNA of 40 F_2

Table 3. Chromosome location, Genebank accession number, and repeated type of the SSR markers used in the tudy.

SSR marker	Chromosome/linkage group	Gene Bank accession number	Repeated type
Satt_070	B2	BH126318	(ATT)24
Satt_406	J	CC453964	(ATT)31
Satt_516	F	BH126686	(ATT)19
Satt_182	L	BH126390	(ATT)17
Satt_144	F	BH126356	(ATT)18

Table 4. Primer sequences of the SSR markers used in the study.

SSR marker	Forward	Reverse
Satt_070	5'-TAAAAATTAAAATACTAGAAGACAAC-3'	5'-TGGCATTAGAAAATGATATG-3'
Satt_406	5'-GCGTGAGCATTTTTGTTT-3'	5'-TGACGGGTTTAATAGCAT-3'
Satt_516	5'-GCGTTAGCACTATTTTTTTACAAGA-3'	5'-GCGCCGTTCCTCTTTACTTTAT-3'
Satt_182	5'-GGTCCACATGAAATGAAGGT-3'	5'-TCTCAGCCTGCAAAGAAAA-3'
Satt_144	5'-CGTCGCCATCACTATGAGAA-3'	5'-CCATCTTGAGCAGAGTTTGAAGTT-3'

individual plants (selected randomly) per population were isolated from the leaf of a two week-old plants. DNA was isolated using a CTAB buffer using the method of Keim *et al*. (1988). DNA quality and quantity were determined as described in the section of F_1 plants confirmation using a standard agarose gel electrophoresis (Sambrook *et al*. 1989). As in the F_1 plant determination studies, we also used the SSR marker Satt_070 in this segregation study as this marker was polymerphic in all parent combinations of the four populations used in this experiment. PCR was conducted as described (Akkaya *et al*. 1995). A *Chi Square test* then was conducted to test a single gene segregation ratio of the tested marker from each population. The null hypothesis of the test was that the progeny segregated in a 1 : 2 : 1 ratio (homozygous to the first parent: heterozygote: homozygous to the second parent). A further study was conducted using four SSR markers distributed across three chromosomes of the soybean genome in 94 progeny of a single population, B3462 X B3293. The four SSR markers used were Satt_406 (LG J), Satt_516 (LG F), Satt_182 (LG L), and Satt_144 (LG F) with the characteristics of each SSR marker as shown in Tables 3 and 4. This study was designed to confirm that no marker skewness should be observed. Seeds derived from each individual F_2 plants were harvested separately.

F_2 plant characterization was conducted at the ICABIOGRAD Experimental Station, Pacet, West Java. Parameters observed include plant height, branch number, seed yield per plant, and weight of 100 seeds. Seed coat color, a classical marker segregating in the progeny of two of the four populations was also observed. This was used to confirm the results of SSR marker segregation ratio.

RESULTS AND DISCUSSION

Glass House Study of 23 Soybean Genotypes using Poor Nutrient Jasinga Acid Soils

Results of this study were in agreement with the genotype designation shown in Table 1 in the sense that genotypes designated as tolerance to Al toxicity in Table 1 were also observed as tolerance in this study. Similarly, the genotypes designated as sensitive in Table 1 were also sensitive in this study (Table 5 and Figure 1). Two tolerant genotypes (B3462 and B3851) always showed superior performance. In contrast, two sensitive genotypes (B3293 and B3442) always showed the inferior phenotypic performance (Table 5 and Figure 1). Based on this study three tolerant and two sensitive genotypes were selected for parents to make a total of four single crosses.

Development and Characterization of F_2 Population Segregating for Al-toxicity Tolerant Character

Four crossing combinations (B3462 X B3293, B3851 X B3293, B3462 X B3442, and B3851 X B3442) were made. The putative F_1 plants were tested their banding patterns using SSR markers. The selected F_1 plants are the ones showing heterozygote DNA bands (i.e., the plants having DNA bands originated from both parents) (Figure 2). For example, in population B3462 X B3293, seven plants (column number 1, 2, 5, 6, 7, 9, and 10) were heterozygotes and, therefore they are true F_1 (Figure 2). Column number 3, 4, and 8, however, were not F_1 because they have DNA bands originated from their female parent only (Figure 2). And these three later plants, therefore, were discarded early in the breeding program and not be included for further studies or breeding steps. This selection system will make the population development be more

Table 5. Growth performances of 23 soybean genotypes on Jasinga acid soils in a glass house condition.

Genotype	Plant height (cm) 50 dap	Al chlorotic[#] score value (30 dap)	Pod number[$] per plant (60 dap)	Seed number per plant (60 dap)
B4408	30.99h	2.0cde	0.76cde	0.24a
B4378	29.75h	0.9a	0.80cde	0.70bcd
B4375	30.73h	1.3abc	0.41ab	0.27a
B4372	18.13ab	2.2de	0.76cde	0.44abc
B3866	27.41efg	2.2de	0.88def	1.03de
B4374	26.72ef	3.4f	0.40ab	0.18a
B4409	19.13b	1.6abcd	0.80cde	0.80bcde
B4410	15.58a	2.0cde	0.87def	0.42ab
B4411	17.12ab	0.9a	0.56abc	0.20a
B4412	16.35a	1.7bcd	0.58abc	0.24a
B4403	32.13i	1.2ab	0.78cde	0.83cde
B3462*	30.25h	0.9a	1.52g	2.62f
B4377	29.54gh	2.2de	0.64bcd	0.28a
B3442**	22.26c	3.6f	0.33a	0.16a
B4368	29.62gh	1.7bcd	0.88def	0.28a
B3293**	25.58de	3.5f	0.44ab	0.28a
B3531	22.85cd	1.6abcd	0.54abc	0.20a
B3614	28.46fgh	2.2de	1.02ef	1.15e
B1958	34.15i	1.8bcd	1.04ef	1.08de
B3776	27.76efg	2.6e	1.14f	1.09de
B4311	22.31c	3.4f	1.12f	1.08de
B3898	27.98efg	1.8bcd	0.35a	0.18a
B3851*	31.05hi	1.2ab	1.47g	2.25f

Numbers followed by the same characters in each column are not significantly different at p = 0.05 of Duncan's Multiple Range Test. [$]Only pods of ≥1 cm sizes with or without seeds were counted. DAS = days after sowing. This study was conducted without applying any fertilizer. * and ** = lines were selected for further studies.

A = B3462 (Tolerant), B = B3293 (Sensitive) to Al toxicity

Figure 1. Phenotypic performances of 60 day-old soybean genotypes showing a contrast tolerance to aluminum toxicity in a glass house condition.

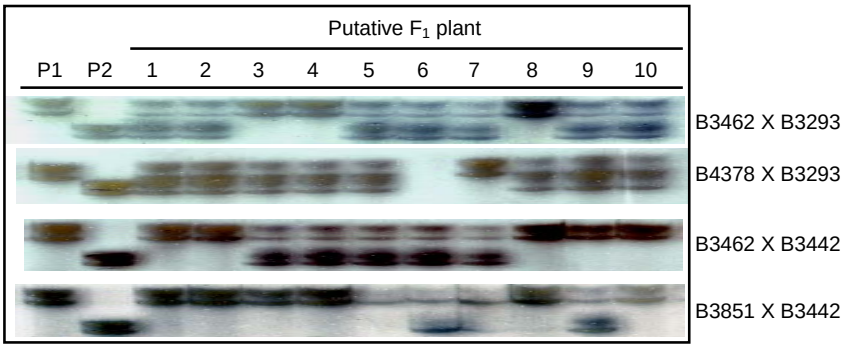
efficient as we save more man power and breeding resources. This further proves the powerfulness and usefulness of the co-dominant SSR marker.

Using SSR marker Satt_070 we found that 50 to 80% of the plants tested were F_1 except for crossing pair of B3851 and B3442 with only 20% of the putative F_1 plants were real F_1 (Figure 2). For QTL mapping purposes only a single F_1 plant is expected to be used for F_2 population development. A single F_1 plant was then selected from each cross ($F_{1\text{-}1}$ for B4362 X B3293, $F_{1\text{-}7}$ for B3462 X B3442, $F_{1\text{-}2}$ for B3851 X B3293, and $F_{1\text{-}9}$ for B3851 X B3442). The selected F_1 plants were self-pollinated to obtain F_2 seeds. The F_2 population sizes were 275 (B3462 X B3293), 388 (B3462 X B3442), 300 (B3851 X B3293), and 325 (B3851 X B3442).

Chi-square test of 40 plants each (selected randomly) per population using SSR marker Satt_070 showed that all four populations followed a 1 : 2 : 1 ratio (Table 6). Similar result was shown by 94 progeny of population B3462 x B3293 using four SSR markers (Table 7) indicating that the alleles from both parents were segregating in the progeny of the populations. This indicates that the tested populations are the segregating F_2 populations. Other evidence is that the

four populations do not show any indication of skewness toward any alleles of either parent.

After two generations of population development, the above conclusion was also supported by the phenotypic data of seed coat color (Figure 3, Table 8). *Chi-square* test showed that seed coat color segregated in a 1 : 2 : 1 (black: green: yellow) for the B3462 X B3293 population and a 3 : 1 ratio (green: yellow) for the B3462 X B3442 population. This finding supports the ones shown by SSR marker Satt_070 indicating that SSR marker is very useful and powerful in the F_2 population development. The brown seed coat color shown in Figure 3A is actually the imperfect black and, therefore, is classified as the black seed coat color (Figure 3). This phenomenon is due the action of gene



P_1 = female parent, P_2 = male parent

Figure 2. Confirmation of F_1 plants from crosses of Al-toxicity tolerant and Al-toxicity sensitive soybean parents using the SSR marker Satt_070.

A = B3462 X B3293, B = B3462 X B3442

Figure 3. Segregation of seed coat color of two F_2 populations segregating for seed coat color.

Table 6. *Chi-Square* test of SSR marker Satt_070 using 40 randomly selected progeny of four soybean populations from crosses between the Al-toxicity tolerant and Al-toxicity sensitive parents.

Population	Segregation						X^{2a}
	Observed ratio			Expected ratio			
	A	H	B	A	H	B	
B3462 X B3293	8	21	11	10	20	10	0.55
B3462 X B3442	9	21	10	10	20	10	0.15
B4378 X B3293	12	20	8	10	20	10	0.80
B3851 X B3442	11	17	12	10	20	10	0.95

[a]The null hypothesis of the test was that progeny segregated in a 1 : 2 : 1 ratio (A:H:B). A = allele was from first parent (B3462, B4378, or B3851), B = allele was from the second parent (B3293 or B3442), H = allele was from both parents.

R causing the variation of seed hylum color and the action of gene *T* for brown pubescent color. Allelic combination, *RT* demonstrated black seed coat color and combination of *rT* showed the brown one (Bernard and Weiss 1973).

Characteristics of two most promising populations (B3462 X B3293 and B3462 X B3442) showed wide transgressiveness (some progeny demonstrated much higher or much lower phenotypic values compared to their parents) for reproductive characters such as pod number and weight of 100 seeds (Table 9). This indicates that there are several other agronomically important traits potentially to be dissected from these populations. One of the above populations (B3462 X B3293) is being used for genotyping using SSR markers to construct an SSR genetic map and for phenotyping in response to Al toxicity both in glass house and in field conditions. Both data will be used to dissect QTL controlling aluminum toxicity tolerant trait of soybean.

Table 7. *Chi-square* test of four SSR markers segregated in 94 progeny of F_2 population B3462 X B3293. There are a total of 200 progeny in this population.

SSR Marker	Segregation						X^{2a}
	Observed ratio			Expected ratio			
	A	H	B	A	H	B	
Satt_406 (LG J)	22	47	21	22.5	45	22.5	0.11
Satt_516 (LG F)	21	39	29	22.25	44.5	22.25	0.82
Satt_182 (LG L)	27	41	22	22.5	45	22.5	1.27
Satt_114 (LG F)	20	53	21	23.5	47	23.5	0.14

[a]The null hypothesis of the tests was that progeny segregated in a 1 : 2 : 1 ratio of which the alleles were derived from the first parent, both parents, and from the second parent, respectively. All four markers followed a 1 : 2 : 1 segregation ratio of a single gene hypothesis. LG = linkage group/chromosome.

Table 8. *Chi-square* tests of seed coat color of populations B3462 X B3293 and B3462 X B3442.

Population	Segregation ratio[a]						X^{2a}
	Observed ratio			Expected ratio			
	B	G	Y	B	G	Y	
B3462 X B3293[*]	52	108	50	50	100	50	0.72
B3462 X B3442[**]	-	186	64	-	187.5	62.5	0.03

[a]B = black, G =g reen, and Y = yellow seed color. [*]The null hypothesis of the test was that progeny segregated in a 1 : 2 : 1 ratio of which the alleles were derived from the first parent, both parents, and the second parent, respectively. [**] The null hypothesis of the test was that progeny segregated in a 3 : 1 ratio of which the alleles were derived from the first parent, and from the second parent, respectively.

Table 9. Phenotypic performance of two F_2 populations grown at BB-Biogen's Pacet Experimental Station, Cipanas, West Java (5 October 2006 to 26 January 2007).

Trait observed	Parent			Population		
	B3462	B3293	B3442	Average	Standard deviation	Min/max[a]
B3462 X B3293 (211 progeny F_2)						
Plant height (cm)	62.7	43.7	-	46.81	10.05	29.3/67.6
Pod number/plant	64.7	118.3	-	110.63	38.60	30/375
Seed yield/plant (g)	25.2	28.2	-	27.6	8.96	13.3/72.4
100 seed weight (g)	18.2	12.1	-	15.51	5.35	12.4/23.2
B3462 X B3442 (260 progeny F_2)						
Plant height (cm)	62.7	-	76.3	60.86	11.06	41.2/90.4
Pod number/plant	64.7	-	116.7	109.74	37.78	42/342
Seed yield/plant (g)	25.2	-	23.2	25.78	8.92	20.7/108.2
100 seed weight (g)	18.2	-	8.5	13.77	4.22	10.4/16.8

[a]Min/max = the range of minimum and maximum values for each trait observed in the F_2 progeny of the two populations.

CONCLUSIONS

Four F_2 populations were developed. The populations segregated in a 1 : 2 : 1 ratio of the alleles originnated from the first, both, and the second parents, respectively. SRR marker increases the speed, accuracy, and efficiency of the F_2 population development. Two selected populations demonstrated high transgressiveness on three agronomically important traits suggesting that other agronomic characters are potentially to be dissected from these populations. Population development methodology presented in this paper will be very useful for soybean Breeders for an efficient breeding program as well as for Geneticists looking for an agronomic character.

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