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MARKER-ASSISTED SELECTION COMBINED WITH CONVENTIONAL BREEDING ON SOYBEAN IN THE MEKONG DELTA

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ABSTRACT

Thirty promising soybean genotypes have been selected through crossing in 2003-2009 at CLRRI. Five polymorphic markers as Satt005, Satt083, LP, SSV, S35 were used in soybean breeding to gain genetic advances on productivity, and disease resistance. Evaluation of 15 promising lines selected from on observational plot showed that all of them exhibited 77-93 days for growth duration and 14-70 cm for plant height. OMDN112, OMDN114, OMDN110, OMDN109, OMDN117 were successfully selected as promising progenies, which exhibited high productivity.

Key words. marker-assisted selection (MAS), soybean

INTRODUCTION

Soybean (*Glycine max* L.) is an important crop plant in worldwide. It has been used in Vietnam as source of vegetable oil and food. The soybean genome has been proposed as a model for phaseoloid legumes due to its economic and biological importance, moderate genome size (1.12-1.81 x 10^9 bp), and its extensive research history (Xia et al. 2007). The cropping pattern of rice - soybean, rice - soybean - rice, rice – soybean - soybean, rice –soybean – aqua-culture have been developed in Mekong delta.

New soybean varieties were become necessary for rotation of rice-soybean in Mekong Delta Old soybean varieties were cultivated too long and degenerated, their yield is low and resistant ability to major diseases and insects were decreased. Cultivated profit from those soybean varieties for the farmers does not have advantage. To widen the gene pool and genetic diversity in breeding program, soybean breeders need to know the genetic background of the germplasms they are using. Characterization of current germplasms, for example, by labeling agronomically valuable traits with molecular markers, is of fundamental importance in this aspect. Reliable molecular marker development and polymorphism analysis of germplasms are plausible first stages in this varietal characterization. Microsatellites, or simple sequence repeats (SSRs), have greater potential in polymorphism analysis and marker assisted selection than other types of molecular markers (Thiel et al. 2003; Jiang et al. 2006). However, the conventional development of SSR markers by SSR enriched genomic library construction and large-scale sequencing is costly and labor intensive (Varshney et al. 2002). The occurrence of SSRs in ESTs provides an opportunity to use currently available ESTs in databases for SSR development (Qiu et al. 2000).

The study aims at clustering soybean genotypes by SSR polymorphic analysis, releasing new high-yielding soybean with short growth duration, resistance to major pests and diseases

MATERIALS AND METHODS

Thirty promising soybean varieties have been selected through crosses during 2003-2009 DNA isolation

Protocol for DNA extraction was done according to the method suggested by Zheng et al. (1995) and modified by Lang (2002). Healthy rice leaf sample (2 cm long) was collected and placed in a labeled 1.5ml centifuge tube on ice. Cut the leaf tissue into 0.5cm long segments and ground in a well of the thick polished glass rod with a small pestle after adding 400µl of extraction buffer

(50mM tris-HCl pH 8.0, 25mM EDTA, 300 mM NaCl and 1% SDS). The tissue was ground until the buffer turns dark green. Added 400µl more of DNA extraction buffer and mixed in the well by pipetting. 400µl of the lysate was transfered to the original 1.5 ml of the leaf sample. Added 400µl chloroform and mixed well by inverting. Spin the tube for 30 sec in microcentifuge. The aqueous supernatant was transfered to a new 1.5 ml tube and DNA precipitated using absolute ethanol. Spin for 3 min at 13.000 rpm and discarded the supernatant. After drying in air, the DNA was resuspended in 50µl of TE buffer (10mM tris-HCl pH 8.0, 1mM EDTA pH 8.0). DNA was done for PCR analysis. Stored DNA at –20^{o}C for later use.

PCR amplification

PCR amplification was performed in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.5 to 1.0 unit of Taq polymerase, 200 µM each of dNTP, 0.25 µM of primer with 10 ng of genomic DNA per 20 µl using a thermalcycler. An initial denaturation was performed at 94oC for 2 minutes prior to 30 cyles of denaturation at 94oC (1 minute), annealing at 55oC (1 minute), and extension at

72oC (2 minutes). A final extension for 5 minutes at 72oC will be performed. Polymorphisms in the PCR products will be detected after electrophoresis on on 3% agarose gel for microsatellite before ethidium bromide staining.

Sequence information of the selected polymorphic SSR primers used

Primers	Sequence 5'----------3'	Allele	Size (bp)
Satt 005	TAT CCT AGA GAA GAA CTA AAA AA GTC GAT TAG GCT TGA AAT A	2	190-200
Satt083	ACC ATT GGA ATG TTC TAC A TTG AAG TTA TAA AAA AGT TTA CAT	3	280-300
S35	'GCTCCTACAAATGCCATCA GATAGTGGGATTGTGCGTCA	2	300-280
SSV	GTAATCT(Ta)ACCACTGTGTGTG TGGTCTCCTTTGGA(AG)GCCCCC-	2	200-220
LP	TATAGCAATGTGTGCGCTGG GTTCCCTTCCAGCAGCTAAC	2	190-160

The experiments were laid out in randomized complete block design (RCBD) with 3 replications, spacing 20 x 40 cm, sowing 3 grains/ hill. Fertilizer dosages: 40 - 60 - 40 kg NPK / ha. Varieties were sown on the well ploughed land. Seeding was done by making a small hole with a wooden tool to have spacing of 30 x 80 cm (plant x row). Pitting two seeds per hole and covering with rice husk ashes. Watering to make suitable humidity for seed germination was conducted. Farming technique was followed by farmers' habits.

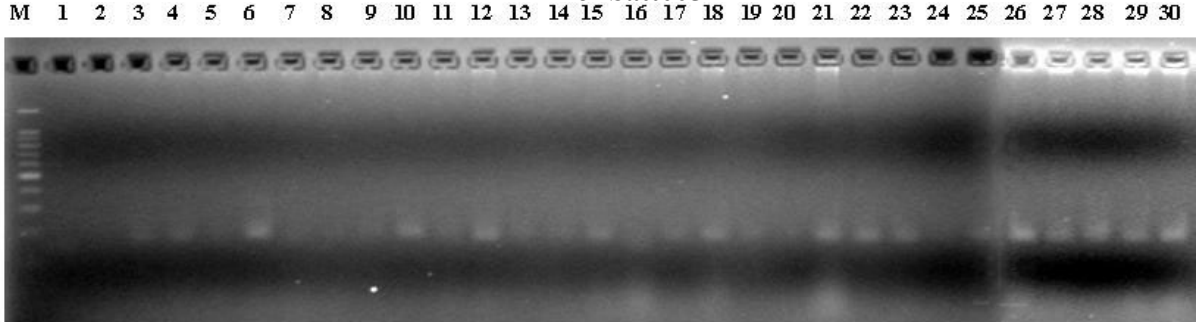
RESULTS AND DISCUSSION

PCR amplification and polymorphism analysis

In total, 30 varieties used in the experiment and five primers detected the banding patterns. They were generated by the respective primers, which can be classified into several types based on the presence or absence of bands of various sizes in the gels. The details of primer sequences, amplified fragment size, annotation (if available) of the SSR sequences and polymorphisms among the tested soybeans are listed in Table 2. Importantly, five pairs of primers were able to detect polymorphisms in the tested soybean varieties (Figure 1). Interestingly, we randomly selected 33 SSRs containing ESTs and designed primers to amplify the SSRs. Of 33 SSRs, twenty-one achieved successful amplification. The twelve instances where amplification was not achieved

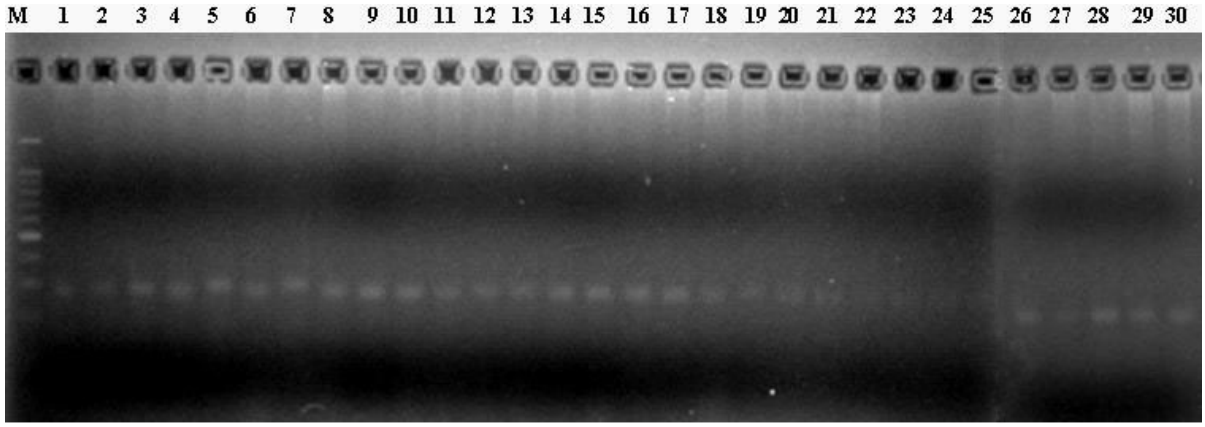
were mainly due to poor primer quality. The length of these SSR ranged from 100 bp to 250 bp. Based on this ratio we could expect to identify more than five SSRs with polymorphisms in different soybean cultivars. The polymorphism screening experiment was undertaken using all SSRs identified in this study. The results of this study will greatly facilitate the study of soybean genetic diversity analysis and marker assisted selection.

Primer Satt005



1/OMDN64	2/ATF15	3/OMDN32	4/OMDN83	5/OMDN31	6/OMDN87
7/NamVang	8/OMDN109	9/DT84	10/OMDN118	11/OMDN116	12/OMDN86
13/OMDN59	14/OMDN115	15/OMDN34	16/OMDN62	17/OMDN111	18/OMDN36
19/OMDN117	20/OMDN14	21/OMDN29	22/OMDN110	23/OMDN114	24/OMDN85
25/OMDN112	26/OMDN33	27/OMDN113	28/MTD176	29/TL57	30/OMDN1

Primer Satt083



1/OMDN64	2/ATF15	3/OMDN32	4/OMDN83	5/OMDN31	6/OMDN87
7/NamVang	8/OMDN109	9/DT84	10/OMDN118	11/OMDN116	12/OMDN86
13/OMDN59	14/OMDN115	15/OMDN34	16/OMDN62	17/OMDN111	18/OMDN36
19/OMDN117	20/OMDN14	21/OMDN29	22/OMDN110	23/OMDN114	24/OMDN85
25/OMDN112	26/OMDN33	27/OMDN113	28/MTD176	29/TL57	30/OMDN1

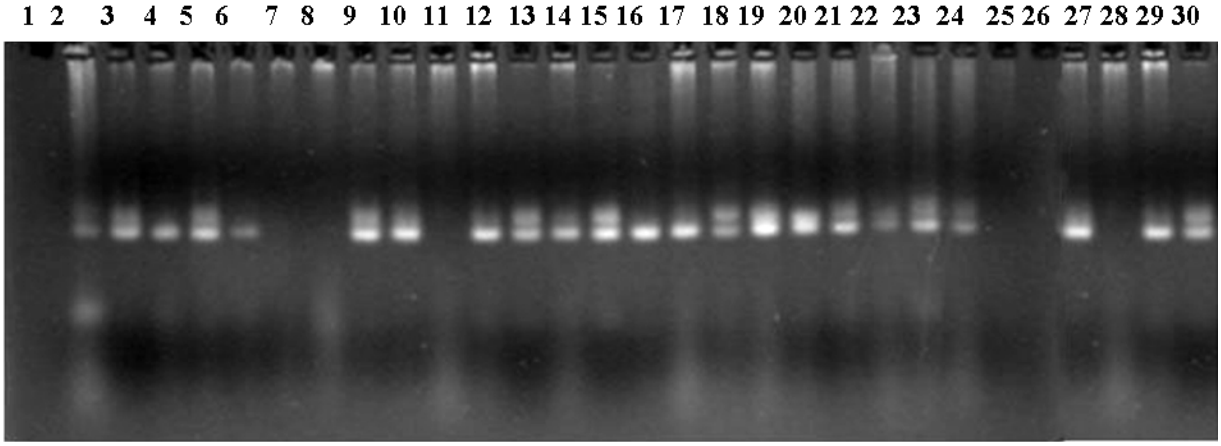


Figure 1. PCR products at the loci LP

Performance trials of soybean varieties

Table 1. Origin of promising varieties

No	Designation	Cross
1	OMDN110	AK05/OMDN29
2	OMDN29	OMDN1/KETTUM
3	OMDN109	Nam vang/HSp3
4	OMDN112	MTD 176/ATF7
5	OMDN111	MTD176/ATF15
6	OMDN117	MTD 652-4/ G10
7	OMDN87	OMDN30/DT93
8	OMDN1	MAS
9	OMDN118	DT 93/ ATF16
10	OMDN85	GC9001-41-5/KETHAM
11	OMDN116	DT84/G20
12	ATF15	From the IAS
13	OMDN114	MTD455-3/OMDN1
14	OMDN113	ĐMT 483-4/ G25-2
15	OMDN115	MTD517-8/ATF15

Table 2. The flowering duration (days)

No	Designation	Flowering duration (days)				
		06-07Dry	07 Wet	08-09Dry	09 Wet	Mean
1	OMDN110	34	36	33	34	34
2	OMDN29	34	31	33	34	33
3	OMDN109	34	34	32	34	34
4	OMDN112	35	34	35	35	35
5	OMDN111	34	36	34	35	35
6	OMDN117	36	40	36	37	37
7	OMDN87	32	32	33	33	33
8	OMDN1	33	31	32	33	32
9	OMDN118	34	33	34	33	34
10	OMDN85	31	32	32	34	32
11	OMDN116	33	31	32	34	33
12	ATF15 (check)	34	31	33	33	33
13	OMDN114	34	39	35	36	36
14	OMDN113	35	37	35	36	36
15	OMDN115	36	31	34	33	34

Evaluation of 15 promising lines selected from on observational plot showed that all of promising lines have growth duration from 77-93 days and plant height from14–70cm.(Table 3, 4).

Table 3. Growth duration (days)

No	Designation	Growth duration (days)				
		06-07Dry	07 Wet	08-09Dry	09 Wet	Mean
1	OMDN110	85	87	87	91	88
2	OMDN29	84	88	88	92	88
3	OMDN109	80	86	86	91	86
4	OMDN112	82	90	83	92	87
5	OMDN111	82	91	85	92	88
6	OMDN117	78	97	77	93	86
7	OMDN87	78	79	80	82	80
8	OMDN1	76	78	80	81	79
9	OMDN118	78	81	78	81	80
10	OMDN85	80	89	90	93	88
11	OMDN116	84	88	85	92	87
12	ATF15 (check)	77	78	79	84	80
13	OMDN114	85	93	90	93	90
14	OMDN113	84	87	85	90	87
15	OMDN115	84	86	86	92	87

Table 4. Plant height (cm)

No	Varieties	Plant Height (cm)				
		06-07Dry	07 Wet	08-09Dry	09 Wet	Mean
1	OMDN110	33.0cd	53.00 abcde	44.6	60b	44.6
2	OMDN29	14.6i	22.07 g	43.6	48efgh	43.6
3	OMDN109	25.2efgh	43.33 ef	33.7	47fgh	33.7
4	OMDN112	26.7ef	49.07 bcde	42.5	51def	42.5
5	OMDN111	26.1efg	50.27 bcde	37.3	51def	37.3
6	OMDN117	34.8bc	49.33 bcde	45.3	50defg	45.3
7	OMDN87	34.2bc	45.27 cdef	40.8	52cdef	40.8
8	OMDN1	22.5hg	44.20 def	42.7	42hi	42.7
9	OMDN118	27.1ef	48.00 bcdef	45.9	47ghf	45.9
10	OMDN85	35.1bc	54.40 abcd	42.6	59bc	42.6
11	OMDN116	38.7ab	57.93 ab	35.9	56bcd	35.9
12	ATF15 (check)	23.5fhg	38.47 f	40.4	44ghi	40.4
13	OMDN114	21.0h	49.40 bcde	43.1	38i	43.1
14	OMDN113	29.7de	55.20 abc	43.6	55bcde	43.6
15	OMDN115	40.5a	62.40 a	37.5	70a	37.5
	CV (%)	11.6	12.9	13.5	16.9	

Table 5. The number of pods / plant

No	Designation	No. of pots / plant				
		06-07Dry	07 Wet	08-09Dry	09 Wet	Mean
1	OMDN110	26.5a	42.27 bc	31.1	54abcdef	31.1
2	OMDN29	12.2d	28.53 de	27.8	70a	27.8
3	OMDN109	23.7ab	44.27 bc	20.9	48def	20.9
4	OMDN112	21.5abc	52.93 b	37.4	63abcd	37.4
5	OMDN111	22.0ab	48.13 bc	20.6	56abcdef	20.6
6	OMDN117	25.5a	66.07 a	31.7	59abcdef	31.7
7	OMDN87	20.3abc	51.87 b	43.4	58abcde	43.4
8	OMDN1	17.6abcd	37.20 cd	36.5	44ef	36.5
9	OMDN118	17.8abcd	48.93 bc	26.4	52cdef	26.4
10	OMDN85	18.01abcd	53.53 b	21.6	67abc	21.6
11	OMDN116	24.3a	38.53 cd	22.1	55abcdef	22.1
12	ATF15 (check)	21.0abc	42.80 bc	27.9	48abc	27.9
13	OMDN114	23.3ab	48.87 bc	22.6	43ef	22.6
14	OMDN113	18.5abcd	45.67 bc	29.3	40f	29.3
15	OMDN115	25.3a	54.07 b	26.3	44ef	26.3
	CV (%)	25.4	16.4	31.8	17.5	

Table 6. Ratio of 3-grain pod

No.	Designation	Ratio 3-grain pod / plant				
		06-07Dry	07 Wet	08-09Dry	09 Wet	Mean
1	OMDN110	26.9b	26.13 bcd	21.4	22.9abc	21.4
2	OMDN29	37.2a	16.30 d	21.7	26.1ab	21.7
3	OMDN109	22.8b	45.91 a	15.7	28.0a	15.7
4	OMDN112	10.8d	29.11 bc	21.7	19.9bcd	21.7
5	OMDN111	14.8bcd	33.31 b	33.9	18.5cde	33.9
6	OMDN117	36.6a	44.31 a	23.4	27.1a	23.4
7	OMDN87	23.1b	28.86 bc	36.1	20.0bcd	36.1
8	OMDN1	13.6cd	33.42 b	24.6	17.7cde	24.6
9	OMDN118	19.9bc	28.26 bc	15.9	17.3cde	15.9
10	OMDN85	20.1bc	25.90 bcd	22.2	15.6de	22.2
11	OMDN116	10.1d	33.38 b	26.5	17.0cde	33.5
12	ATF15 (check)	18.7bc	18.41 cd	29.0	17.6bcd	29.0
13	OMDN114	22.0cd	33.52 b	25.2	21.8e	25.2
14	OMDN113	20.8cd	29.19 bc	21.5	16.0cde	21.5
15	OMDN115	12cd	25.25 bcd	22.8	12.4e	22.8
	CV (%)	22.9	20.7	27.5	20.9	

Table 7. Ratio of unfilled pod / plant

No	Designation	Ratio of unfilled pot / plant				
		06-07Dry	07 Wet	08-09Dry	09 Wet	Mean
1	OMDN110	4.6abc	0.80 d	2.8	2.3d	2.8
2	OMDN29	4.4abc	5.89 ab	3.0	3.5cd	3.0
3	OMDN109	6.5abc	1.30 cd	5.2	3.1cd	5.2
4	OMDN112	4.3abc	1.15 cd	4.0	4.8bcd	4.0
5	OMDN111	3.6abc	1.62 cd	2.8	2.9cd	2.8
6	OMDN117	5.0abc	3.30 bcd	4.8	4.3bcd	4.8
7	OMDN87	5.7abc	2.35 bcd	11.3	4.7bcd	11.3
8	OMDN1	6.8abc	3.09 bcd	3.5	6.5ab	3.5
9	OMDN118	3.4bc	2.01 cd	5.8	7.6a	5.8
10	OMDN85	3.8abc	1.92 cd	4.3	4.9abc	4.3
11	OMDN116	7.9a	1.39 cd	2.3	6.8ab	2.3
12	ATF15 (check)	4.4abc	4.44 abc	5.0	4.5bcd	5.0
13	OMDN114	4.9abc	2.02 cd	3.3	4.8bcd	3.3
14	OMDN113	4.0abc	3.76 abcd	4.5	5.3abc	4.5
15	OMDN115	4.5abc	2.36 bcd	2.9	6.3ab	2.9
	CV (%)	23.4	25.1	30.7	34.4	

Table 8. 100-grain weight

No.	Designation	100-grain weight				
		06-07Dry	07 Wet	08-09Dry	09 Wet	Mean
1	OMDN110	14.9	16.3	18.29	17.5	16.7
2	OMDN29	17.4	22.7	17.44	18.3	19.0
3	OMDN109	12.3	15.4	17.74	15.8	15.3
4	OMDN112	15.7	18.0	16.11	18.4	17.1
5	OMDN111	15.3	18.8	14.79	18.7	16.9
6	OMDN117	9.8	13.1	12.21	13.6	12.2
7	OMDN87	13.8	13.5	11.76	13.7	13.2
8	OMDN1	16.5	16.9	15.29	16.8	16.4
9	OMDN118	10.4	12.7	18.14	12.9	13.5
10	OMDN85	17.4	17.5	17.26	17.6	17.4
11	OMDN116	17.3	20.8	14.11	16.4	17.2
12	ATF15 (check)	14.2	15.9	14.00	15.9	15.0
13	OMDN114	13.8	17.6	16.25	17.6	16.3
14	OMDN113	16.4	14.9	16.63	14.6	15.6
15	OMDN115	15.8	19.6	16.00	18.4	17.5

Evaluation on yield and yield components of 15 promising lines and ATF15 (check) revealed that OMDN112 and OMDN114 obtained the best figures as compared to check (table 9)

Table 9. Yield (t/ha)

No.	Designation	Yield (t / ha)				
		06-07Dry	07 Wet	08-09Dry	09 Wet	Mean
1	OMDN110	2.39a	2.86 a	1.965	2.93a	1.97
2	OMDN29	1.53ef	1.34 g	1.586	2.93a	1.59
3	OMDN109	2.03abc	2.39 cd	1.929	2.33ab	1.93
4	OMDN112	2.08 ab	2.99 a	2.238	2.26b	2.24
5	OMDN111	2.18ab	2.70 abc	1.568	2.24b	1.57
6	OMDN117	1.90bcde	1.94 ef	1.815	2.19b	1.82
7	OMDN87	2.10ab	2.49 bcd	1.333	2.17b	1.33
8	OMDN1	1.95bcd	2.40 cd	1.241	2.06b	1.24
9	OMDN118	1.67cdef	1.77 f	1.789	1.96b	1.79
10	OMDN85	1.92bsd	2.87 a	1.768	1.76bc	1.77
11	OMDN116	1.94bcd	2.78 ab	1.705	1.22cd	1.71
12	ATF15 (check)	1.65cdef	2.19 de	1.905	1.19cd	1.91
13	OMDN114	2.03abc	2.95 a	2.104	1.14d	2.10
14	OMDN113	2.03abc	2.48 bcd	1.244	1.12d	1.24
15	OMDN115	1.94bcd	2.50 bcd	1.336	1.06d	1.34
	CV (%)	13.2	9.4	22.1	18.9	

Table 10. Some important morphological characters

No	Designation	Flower color	Pod color	Grain color
1	OMDN110	Violet	Light brown yellow	straw yellow
2	OMDN29	Violet	Yellow	Light yellow
3	OMDN109	Violet	Yellow	straw yellow
4	OMDN112	Violet	Yellow	straw yellow
5	OMDN111	Violet	Yellow	straw yellow
6	OMDN117	Violet	Light brown yellow	straw yellow
7	OMDN87	Violet	Yellow	straw yellow
8	OMDN1	Violet	Yellow	straw yellow
9	OMDN118	Violet	Yellow	yellowish
10	OMDN85	Violet	Yellow	straw yellow
11	OMDN116	Violet	Yellow	straw yellow
12	ATF15 (check)	Violet	Yellow	straw yellow
13	OMDN114	Violet	Yellow	straw yellow
14	OMDN113	Violet	Light brown yellow	yellowish
15	OMDN115	Violet	Yellow	straw yellow

CONCLUSIONS

- SSR marker were tested as a means of positively identifying each of 30 soybean by CLRRI. Six SSRs showed polymorphism in banding pattern, which distinguished among the cultivars. The relationships among the 30 cultivars was analyzed by similarity values and a dendrogram constructed (not given in the text). Three distinct groups were recognized among 30 varieties.
- During four seasons, the varieties were evaluated in multi-locational trials. We realized that most of them are promising soybean varieties. Especially, five varieties as: OMDN112, OMDN114, OMDN110, OMDN109, OMDN117 obtained high and stable yield. They should be released soon

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Chọn tạo giống đậu nành bằng marker phân tử kết hợp với phương pháp truyền thóng ở đồng bằng sông Cửu Long

Ba mươi dòng đậu nành được chọn lọc từ các tổ hợp lai trong thời gian năm 2003-3007 tại Viện Lúa ĐBSCL. Năm primer như: Satt005, Satt083, LP, SSV, S35 đã được sử dụng để chọn lọc kiểu gen các dòng đậu nành có tiềm năng về năng suất và khả năng kháng bệnh cao. Kết quả đã đánh giá được mười lăm dòng đậu nành triển vọng, có thời gian sinh trưởng từ 77-93 ngày, cao cây từ 12-70 cm. Đã chọn được các dòng đậu nành OMDN112, OMDN114, OMDN110, OMDN109, OMDN117 mang gen tiềm năng về năng suất cao.