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Evaluation of Genetic Characteristics of Introduced Mung Bean Varieties Based On Agronomic Traits and SSR Markers

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Abstract: The aim of this research is to evaluate the genetic characteristics of 9 introduced mung bean varieties, thereby creating a database of agronomic characteristics, as a basis for future studies. The experiment was conducted from January to April at the net house of the School of Agriculture and the Laboratory of Molecular Biology, Institute of Food and Biotechnology, Can Tho University, Vietnam. The experiment was arranged in a completely randomized design with 1 factor, 9 treatments and 4 repetitions, in which the Taichung variety was selected as the control variety. The results showed that the mung bean varieties had two harvests and grew well. The seed shape of introduced varieties was diverse with the highest Shannon index (1.08). Besides, mung bean DNA was amplified with 3 sets of SSR primers (CEDG026, CEDG232, CEDG037) of linkage groups of 2, 4, and 6, respectively. The results showed that all 3 primer pairs appeared in monomorphic bands. The broad-sense heritability of traits varied from 0.02% to 63.60% in first harvest and 33.32% to 78.41% in second harvest. Moreover, the correlation coefficient results showed a strong positive relationship between the yield and the number of pods per plant. Based on important agronomic traits including the number of pods per plant, 1000 seed weight, growth time and yield, 2 promising mung bean varieties were initially selected (VC 6494-986-S7 and VC 6518-50), of which VC 6494-986-S7 had superior yield compared to the control variety, used as a material for further studies.

Keywords: agronomy; genetic characteristics; introduced varieties; mung bean; Simple Sequence Repeat; *Vigna radiata*

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1. Introduction

The mung bean [*Vigna radiata* (L.) Wilczek], a legume that was once only farmed in Asia, has expanded to countries all over the world due to its many uses. As a crucial crop for the economy, mung bean is usually cultivated intercropping with diverse cereals because it increases the nitrogen and carbon availability in the soil for subsequent crops [1]. Mung bean is prized for its great nutritional value because it contains between 20 and 25 percent protein [2]. However, the yield of mung beans in the Mekong Delta in general is still low, averaging 1 ton/ha. Many factors affect this plant's yield, such as cultivation techniques, varieties, climate, soil, etc. One of them is variety, which plays the most important role. Therefore, to achieve the goal of increasing mung bean yield, selecting varieties with high yield and resistance to some pests is necessary [3].

Introduced variety has many important roles such as adding valuable genetic resources, increasing genetic diversity, and serving as a starting material to create new varieties [4]. Diversity in plant genetic resources provides the opportunity for plant breeders to develop new cultivars with desirable characteristics (high yield, large seed, pest and disease resistance, etc.). Hence, the evaluation of genetic characteristics is one of the primary goals of any crop improvement program [5].

Currently, genetic engineering has become one of the effective tools in plant breeding. Genotyping analysis by using DNA markers has been strongly developed, which is one of the techniques to make breeding methods faster and more effective [6]. Because of these reasons, this study: "**Evaluation of genetic characteristics of 9 introduced mung bean varieties based on agronomic traits and SSR markers**" was carried out. The aim of this study is for the genetic characteristics of 9 mung bean varieties to be evaluated based on agronomic traits and SSR markers. From there, 1 or 2 promising varieties could be selected for further research.

2. Materials and Methods

2.1. Materials

Table 1. List of 9 introduced mung bean varieties.

Code	Variety Name	Origin
1	VC 6512-6A	AVRDC, Thailand
2	VC 6570-157-7	AVRDC, Thailand
3	VC 6494-986-S7	AVRDC, Thailand
4	VC 6518-5	AVRDC, Thailand
5	VC 6495-32	AVRDC, Thailand
6	VC 6493-44-7	AVRDC, Thailand
7	VC 6469-12-3-4A	AVRDC, Thailand
8	VC 6469-12-4A	AVRDC, Thailand
9	Taichung (Control)	AVRDC, Taiwan

2.2. Apparatus, equipment and chemicals

Apparatus and equipment used in the study include plant pods, micropipettes, micropipette tips, microcentrifuge tubes, microcentrifuge machine, electrophoresis apparatus, microwave, PCR tubes, PCR Bio-Rad C1000 machine and BIORAD UV 2000 Gel Doc System, etc. Chemicals for DNA extraction include proteinase K, CTAB, β-mercaptoethanol, chloroform/isoamyl alcohol, RNAse, etc. DNA templates, distilled water, PCR Master Mix, agarose, TBE, safe view, loading dye were used for electrophoresis and PCR.

2.3. Methods

2.3.1. Experimental design

Nine varieties were grown in the net house with a completely randomized design (CRD), with 4 replications each. The criteria were based on the International Board for Plant Genetic Resources (IBPGR) [7].

2.3.2. DNA analysis

DNA was extracted from young leaves by an improved CTAB procedure based on the procedure of Doyle, et al. [8] and was modified which is suitable for mung bean by Dharajiya, et al. [9]. The PCR (Polymerase Chain Reaction) component for a total volume of 10 µL included 5 µL of PCR Master Mix; 3 µL PCR water; 0.5 µL forward primer; 0.5 µL reverse primer and 1 µL DNA sample. Everything was mixed well before putting it into the PCR machine. The reaction was carried out in 35 heating cycles, including 5 minutes at 95°C, 30 seconds at 95°C, the next 30 seconds depending on the primer temperature of each SSR primer that was adjusted on the machine accordingly. The chain reaction was carried out for 30 s at 72°C, 5 min at 72°C and the product was stored at 10°C for 20 minutes.

Table 2. List of SSR primers.

Name	Repeat	Primer Sequence	Linkage Group
CEDG026 [1]	$(AG)_{16}$	F: TCAGCAATCATCATGTGGG R: TGGGACAAAC-CTCATGGTTG	2
CEDG232 [2]	$(AG)_{16}$	F: GATGACCAAGGTAACGTG R: GGACAGATCCAAAACGTG	4
CEDG037 [2]	$(AG)_{16}AC(AG)_8$	F: GAAGAAGAACCCTAC-CACAG R: CACCAAAAAC-GTTCCCTCAG	6

[1] Wang, et al. [10]; [2] Dikshit, et al. [11].

2.4. Statistical analysis

The collected data were analyzed for variance (ANOVA) by Minitab 16 and for correlation coefficient by SPSS software. Besides, the Turkey test was used to test the mean difference between mung bean varieties at 1% and 5% significance levels. Processing raw data and calculating statistical characteristics such as mean, coefficient of variation, etc. using Microsoft Excel 2013 software.

3. Results

3.1. Morphological traits

Table 3. Summary table of morphological traits.

Code	Traits	Characteristic	Variety	Shannon index
1	**Hypocotyl color**	Purple	G2	0.48
		Green	G1, G3, G4, G5, G6, G7, G8, G9	
2	**Seed shape**	Oval	G1, G2, G5, G7	1.08
		Cylindrical	G3, G4, G6, G8	
		Other	G9	
3	**Pod color**	Black	G1, G3, G4, G5, G6, G7, G8, G9	0.2
		Brown	G2	
4	**Flower color**	Yellow	All	

Note: G1: VC 6512-6A, G2: VC 6570-157-7, G3: VC 6494-986-S7, G4: VC 6518-5, G5: VC 6495-32, G6: VC 6493-44-7, G7: VC 6469-12-3-4A, G8: VC 6469-12-4A, G9: Taichung.

3.2. Agronomic and yield traits

Table 4. Descriptive statistics of 9 varieties in the first harvest.

Variety	X1	X2	X3	X4	X5	X6
VC 6512-6A	72.65 ^{ab}	77.43 ^{abcd}	6.62 ^{bc}	1.37 ^{c}	6.81 ^{a}	0.34 ^{bcd}
VC 6570-157-7	68.81 ^{b}	73.68 ^{d}	5.56 ^{c}	1.62 ^{bc}	6.62 ^{a}	0.30 ^{d}
VC 6494-986-S7	75.26 ^{ab}	86.48 ^{ab}	7.18 ^{ab}	1.56 ^{bc}	7.68 ^{a}	0.43 ^{ab}
VC 6518-5	74.01 ^{ab}	88.43 ^{a}	8.12 ^{a}	2.31 ^{ab}	7.81 ^{a}	0.45 ^{a}
VC 6495-32	65.21 ^{b}	72.53 ^{d}	6.93 ^{ab}	3 ^{a}	6.37 ^{a}	0.34 ^{bcd}
VC 6493-44-7	71.8 ^{ab}	83.75 ^{abcd}	7.81 ^{ab}	1.93 ^{bc}	6.93 ^{a}	0.36 ^{abcd}
VC 6469-12-3-4A	67.76 ^{b}	74.35 ^{cd}	7.31 ^{ab}	1.62 ^{bc}	6.18 ^{a}	0.31 ^{cd}

VC 6469-12-4A	66.84 ^{b}	75.79 ^{bcd}	7.43 ^{ab}	2 ^{bc}	6.12 ^{a}	0.32 ^{cd}
Taichung (Ctrl)	80.43 ^{a}	86.05 ^{abc}	6.62 ^{bc}	1.68 ^{bc}	7 ^{a}	0.41 ^{abc}
$\overline{X}$	71.42	79.84	7.07	1.9	6.84	0.36
CV%	12.3	12.39	15.92	52.85	36.53	37.33
Min	65.22	72.53	5.56	1.38	6.13	0.30
Max	80.43	88.43	8.13	3	7.81	0.45
$\mathbf{V_p}$	36.72	53.21	0.69	0.32	1.41	0.006
$\mathbf{V_g}$	15.01	29.03	0.44	0.14	1.46	0.005
$\mathbf{V_e}$	21.71	24.18	0.25	0.18	0.0004	0.001
PCV	8.48	9.13	11.80	30.06	17.4	22.07
GCV	5.42	6.74	9.41	22.53	0.29	10.30
$\mathbf{h^2_b}$	40.87	54.56	63.60	56.16	0.02	21.79

Note: X1: Plant height at flowering in first harvest (cm); X2: Plant height at harvesting in first harvest (cm); X3: Number of internodes in first harvest (internode); X4: Number of branches in first harvest (branch); X5: Numer of pods/plant in first harvest (pod); X6: Theoretical yield in first harvest (ton/ha). $\bar{X}$: Mean; CV%: Coefficient of variation; Vp: Phenotypic variance; Vg: Genotypic variance; Ve: Environmental variance; PCV: Phenotypic coefficient of variance; GCV: Genotypic coefficient of variance; h^2b: Heritability in broad sense. Means that do not share a letter are significantly different.

Table 5. Descriptive statistics of 9 varieties in the second harvest.

Variety	X1	X2	X3	X4	X5	X6
VC 6512-6A	77.43 ^{abcd}	82.38 ^{abc}	10.25 ^{c}	2.06 ^{ab}	6.31 ^{ab}	0.28 ^{abc}
VC 6570-157-7	73.68 ^{d}	79.69 ^{c}	8.56 ^{d}	2.43 ^{a}	7.81 ^{a}	0.34 ^{ab}
VC 6494-986-S7	86.48 ^{ab}	93.4 ^{ab}	10.81 ^{bc}	2.25 ^{ab}	8.37 ^{a}	0.44 ^{a}
VC 6518-5	88.43 ^{a}	94.95 ^{a}	12.31 ^{a}	2.37 ^{a}	7.75 ^{a}	0.36 ^{ab}
VC 6495-32	72.53 ^{d}	78.37 ^{c}	10.5 ^{bc}	2 ^{ab}	3.93 ^{b}	0.15 ^{c}
VC 6493-44-7	83.75 ^{abcd}	89.93 ^{abc}	11.62 ^{ab}	1.62 ^{ab}	5.81 ^{ab}	0.24 ^{bc}
VC 6469-12-3-4A	74.35 ^{cd}	80.46 ^{bc}	10.68 ^{bc}	1.44 ^{ab}	5.87 ^{ab}	0.23 ^{bc}
VC 6469-12-4A	75.79 ^{bcd}	82.3 ^{abc}	10.43 ^{c}	1.5 ^{ab}	5.62 ^{ab}	0.23 ^{bc}
Taichung (Ctrl)	86.05 ^{abc}	94.32 ^{a}	10.37 ^{c}	1.18 ^{b}	6.75 ^{ab}	0.36 ^{ab}
$\overline{X}$	79.84	86.2	10.61	1.87	6.47	0.29
CV%	12.39	12.5	14.34	43.18	47.08	52.55
Min	72.53	78.38	8.56	1.19	3.94	0.15
Max	88.43	94.95	12.31	2.44	8.38	0.44
$\mathbf{V_p}$	53.21	65.46	1.11	0.34	3.35	0.01
$\mathbf{V_g}$	29.03	33.72	0.87	0.14	1.11	0.005
$\mathbf{V_e}$	24.18	31.74	0.24	0.2	2.23	0.005
PCV	9.14	9.38	9.93	31.83	28.29	36.12
GCV	6.75	6.73	8.79	20.40	16.33	25.74
$\mathbf{h^2_b}$	54.56	51.51	78.41	41.06	33.32	50.78

Note: X1: Plant height at flowering in second harvest (cm); X2: Plant height at harvesting in second harvest (cm); X3: Number of internodes in second harvest (internode); X4: Number of branches in second harvest (branch); X5: Numer of pods/plant in second harvest (pod); X6: Theoretical yield in second harvest (ton/ha). $\bar{X}$: Mean; CV%: Coefficient of variation; Vp: Phenotypic variance; Vg: Genotypic variance; Ve: Environmental variance; PCV: Phenotypic coefficient of variance; GCV: Genotypic coefficient of variance; h^2b: Heritability in broad sense. Means that do not share a letter are significantly different.

Table 6. Growth time and 1000 seed weight of 9 varieties.

	Growth time at the first harvest (day after sowing)	Growth time at the first harvest (day after sowing)	1000 seed weight (g)
VC 6512-6A	54	79	66.77
VC 6570-157-7	53	78	77.53
VC 6494-986-S7	54	79	75.46
VC 6518-5	54	79.3	72.56
VC 6495-32	55	80.3	71.08
VC 6493-44-7	53	78	72.87
VC 6469-12-3-4A	54	79.3	67.61
VC 6469-12-4A	54	79	68.74
Taichung (Ctrl)	54	79	65.29
Mean	53.88	78.98	70.88
CV%	1.11	0.88	5.8

Note: CV%: Coefficient of variation.

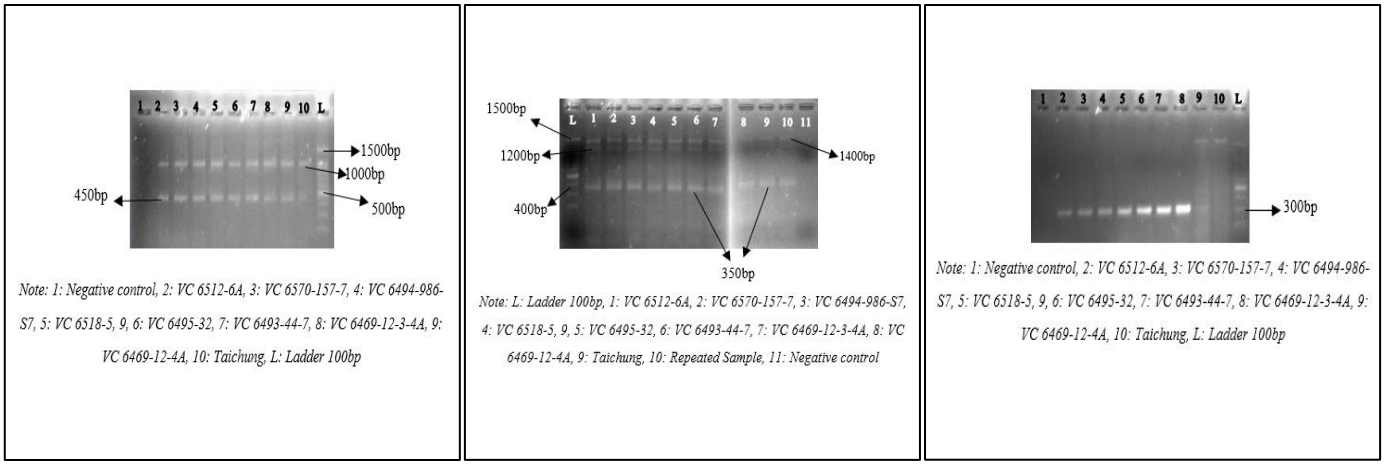
3.3. Correlation coefficient

Table 7. This is a table. Tables should be placed in the main text near to the first time they are cited.

	X1	X2	X3	X4	X5
X1	1				
X2	0.695 *	1			
X3	0.917 **	0.785 **	1		
X4	0.917 **	0.785 **	1.000 **	1	
X5	0.071 ns	0.335 ns	0.357 ns	0.357 ns	1

Note: X1: Theoretical yield in first harvest (ton/ha); X2: Theoretical yield in second harvest (ton/ha); X3: Numer of pods/plant in first harvest (pod); X4: Numer of pods/plant in second harvest (pod); X5: 1000 seed weight (g). (ns): Correlation is not significant; (*): Correlation is significant at 5%; (**): Correlation is significant at 1%.

3.4. DNA analysis



Note: 1: Negative control, 2: VC 6512-6A, 3: VC 6570-157-7, 4: VC 6494-986-S7, 5: VC 6518-5, 9, 6: VC 6495-32, 7: VC 6493-44-7, 8: VC 6469-12-3-4A, 9: VC 6469-12-4A, 10: Taichung, L: Ladder 100bp

(a)

Note: L: Ladder 100bp, 1: VC 6512-6A, 2: VC 6570-157-7, 3: VC 6494-986-S7, 4: VC 6518-5, 9, 5: VC 6495-32, 6: VC 6493-44-7, 7: VC 6469-12-3-4A, 8: VC 6469-12-4A, 9: Taichung, 10: Repeated Sample, 11: Negative control

(b)

Note: 1: Negative control, 2: VC 6512-6A, 3: VC 6570-157-7, 4: VC 6494-986-S7, 5: VC 6518-5, 9, 6: VC 6495-32, 7: VC 6493-44-7, 8: VC 6469-12-3-4A, 9: VC 6469-12-4A, 10: Taichung, L: Ladder 100bp

(c)

Figure 3. DNA analysis results after running on 1% agarose gel electrophoresis. (a) Primer CEDG026; (b) Primer CEDG232; (c) Primer CEDG037.

4. Discussion

4.1. Morphological traits

There were 2 colors of hypocotyl which were green and purple. Out of the total of 9 observed cultivars, 1 cultivar with purple hypocotyl (11.1%) was VC 6570-157-7 and the remaining 8 cultivars had green hypocotyl (88.9%). Besides, the results recorded in 9 experimental varieties all had yellow flowers, the flower sizes of 9 varieties were the same.

The color of ripe pods was recorded: there were 8 varieties had ripe pod with black color: VC 6512-6A, VC 6494-986-S7, VC 6518-5, VC 6495-32, VC 6493-44-7, VC 6469 -12-3-4A, VC 6469-12-4A, Taichung, the Shannon index was 0.2; the remaining variety had ripe pod with brown color was VC 6570-157-7. Seed shape was more diverse and the Shannon index was 1.08, specifically, there were 4 varieties with an oval shape, 4 varieties with a cylindrical shape, and 1 variety with other shapes.

4.2. Agronomic and yield traits

The results of heritability analysis in the broad sense (h^2b) in Table 4 showed that the traits of plant height at flowering in first harvest, plant height at harvesting in first harvest, number of internodes in first harvest, number of branches in first harvest had an average heritability ranged from 40.87% to 63.60%. In addition, traits such as number of pods per plant in first harvest (0.02%) and theoretical yield in first harvest (21.79%) had low heritability.

In Table 5, the results of heritability analysis in the broad sense (h^2b) showed that the trait number of internodes in second harvest had the highest heritability (78.41%). The remaining characteristics had heritability in the broad sense assessed at an average significance level, ranging from 33.32% to 54.56%. From the results of the analysis, we can see that the genetic characteristics in second harvest of the varieties were affected by the environment as in first harvest. Therefore, the investigated genetic characteristics of the varieties are greatly influenced by the environment. Thus, it is necessary to pay attention to the selection of appropriate seasons and proper cultivation techniques to maximize the potential of the variety.

If based on the weight of 1000 seeds, the varieties VC 6570-157-7, VC 6494-986-S7, VC 6518-5, and VC 6493-44-7 were the varieties with outstanding advantages. If the variety would be selected for the purpose of shortening the growth time, the variety VC 6570-157-7 and VC 6493-44-7 were the two suitable varieties (Table 6). However, with the short growth time, the yield of the variety is not high. The yield of 9 varieties showed that VC 6494-986-S7 and VC 6518-5 were suitable choices (Table 4 and 5). Through the evaluation results, the varieties VC 6494-986-S7 and VC 6518-5 were two varieties with outstanding advantages. The varieties were selected despite having an average growth time, but this could be a suitable trait to ensure yield and increase crop intercropping.

4.3. Correlation coefficient

The results in Table 7 showed that the theoretical yield and the number of pods/plant in the first harvest had a strong positive relationship ($r = 0.917^{**}$). The correlation between the theoretical yield and the number of pods/plant in the second harvest also had a strong positive relationship ($r = 0.785^{**}$). It can be seen that the number of pods/plant is one of the important factors affecting mung bean yield, consistent with the evaluation of Thuy, et al. [12]. Whereas, a weak positive correlation was found between 1000 seed weight and theoretical yield in the first harvest ($r = 0.071^{ns}$) as well as theoretical yield in the second harvest ($r = 0.335^{ns}$).

4.4. DNA analysis

The electrophoresis results of PCR products using primer CEDG026 showed a band size of about 450bp. The presence of a band size of 1000bp may be due to mispairing. Whereas, the results of electrophoresis of PCR products using primer CEDG232 showed a band size of about 350bp. Similar to primer CEDG026, the presence of bands at 1200bp and 1400bp may be due to mispairing. Another point is that the electrophoresis results of PCR products using primer CEDG037 showed a band size of about 300bp and a monomorphic band. Thus, the PCR results with primer CEDG026, CEDG232, and CEDG037 showed that the mung bean lines used in the experiment had no change in genotype compared with Taichung (control variety) and were homozygous for date of flowering and weight of 1000 seeds.

5. Conclusions

The data of genetic characteristics of 9 new introduced varieties were established. The results showed that the varieties had 2 harvests, the seed shape trait was diverse with a Shannon index of 1.08. According to analysis results of the characteristics, two promising mung bean varieties, VC 6494-986-S7 and VC 6518-5, were selected. The number of internodes on second harvest had a higher heritability than the remaining traits, which was less influenced by the environment and mainly controlled by genes. The number of pods/plant had a positive correlation with yield, which were r = 0.917 in first harvest and r = 0.785 in second harvest. Therefore, this trait should also be noticed in mung bean yield improvement. Electrophoresis results of PCR of 3 sets of primers showed that all primers gave monomorphic band results. The selected promising varieties should be used for the next studies and should be planted in different geographical areas to test the adaptability to the environment and the stability of the variety. In addition, it is advisable to investigate more primers that cover chromosomes or linkage groups at many different loci.

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