International Journal of Environment, Agriculture and Biotechnology
Vol-8, Issue-5; Sep-Oct, 2023
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Genetic Diversity 20 Bean Varieties using Microsatellite Technique (SSR)

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Received: 02 Sep 2023; Received in revised form: 03 Oct 2023; Accepted: 09 Oct 2023; Available online: 17 Oct 2023

Abstract— *Agro-morphological characters and PCR based markers have provided valuable information about genetic diversity of bean collection in HATRI . Analysis on SSR molecular markers: out of a total of 44 primers conducted genetic diversity studies, only 28 primers amplified the product on 20 bean varieties. Through the SSR marker data with 28 primers used, 20 varieties are classified into 4 main groups. In the subgroup of the SSR on 28 molecular markers are noted with 4 distinct groups. Molecular markers to be able to indirectly assess the presence or absence of selected genes thanks to markers without environmental influences. The diversity index analyzes according to the high SSR method (H = 0.384) while the diversity index of stick beans. The results presented here are the first steps towards a better understanding of varieties introduced from countries and may help guide future research into the crop*.

Keywords— ***bean , gene source diversity, SSR.***

I. INTRODUCTION

The world is facing food insecurity due to climate change and nearly 800 million people from developing countries go to bed hungry (Khush et la.,2012). The world's population is growing rapidly and is estimated to reach 10 billion people by 2050. Therefore, it is necessary to increase world food production by 60%–110% to meet projected food demand by 2050 (Tilman et al.,2011). To meet global food demand, it is necessary to harness plant genetic diversity. Characterization of the gene pool is a strategy in this regard as it helps to discover genotypic and phenotypic variants that can be effectively selected by the breeding community for use (Nadeem et al., 2020, Baloch et al.,2017). Almost 41,500 gene sources from the genus *Phaseolus* are present at the International Center for Tropical Agriculture (CIAT) (Islam, et al., 2006); In addition, there are hundreds of local varieties present in the fields of farmers in bean-growing countries. Most of the genes available at gene resource centers breed quite race.Gene pool characterization has always been one of scientists' favorite methods of investigating new variants that can be used to develop improved plant varieties that exhibit higher yields with better quality, bio-stress resistance and abiotics (Nadeem et al., 2020, Nadeem et al.,2018,). A large number of studies have been conducted to explain morphological, phenomenological and agronomic variation among local bean populations in parts of the world (Boros et al.,2014; Madakba et al.,2011). Rana et al.,2015 explored agronomic and morphological variations in the bean variety and proposed several genotypes that work well for a breeding perspective and (Bozo et al.,2011) used the Turkish bean variety to explore phenotypic variations and report the existence of a variety of phenotypic diversity. Characterization and evaluation of diversity among traditional varieties will provide plant breeders information necessary in the identification of initial materials for hybridization to produce varieties with improved productivity and quality.The objectives of the study are: to evaluate genetic diversity of the bean varieties in the genebank of High Agricultural Technology Research Institute of Mekong Delta, CanTho (HATRI), Vietnam using morphological characters and microsatellite markers. To study correlation among the characters for

application in plant breeding, and and to relate results between morphological characters and molecular markers.

II. MATERIAL AND METHOD

The materials used include 20 bean seed samples collected at the gene bank of the HATRI. The experiment was conducted at the High Agricultural technology Research Institute of Mekong Delta (HATRI). Morphological and agronomic characterization of imported pea gene banks and selected plant varieties are carried out according to plant variety evaluation criteria developed by the International Institute of Plant Genetic Resources (IPGRI 1982) and European Community Plant Varieties (EU-CPVO), (2013). All characteristics are measured on 10 representative individual plants, for 1 variety. A total of 10 seeds were randomly selected, fully developed and undamaged. Ten plants of each genotype were sown, with a distance of 30 cm X 30 cm, completely randomized block design (CRBD) with three iterations. All characteristics are measured on 10 representative individual plants, for 1 variety. The qualitative characters differ as measured according to the description IPGRI (1986) for beans.A total of 10 randomly selected, fully developed and seeds were used to measure seed length, width, and height using a digital Vernier caliper. Root length (cm) was measured by following the methodology of Aghamir et al 2016, (8) Width of fruit, (9) number of fruits per string, (10) number of seeds per tree (11) Yield of fruit (kg) and (12) Yield of hectares (tons/ha).

DNA extraction The DNA molecule for PCR analysis is prepared according to the simplified miniscale process. A sample of fresh, young leaves (2 cm) was collected and placed in a test tube, centrifuged 1.5ml with markings, in ice. The leaves are ground in a mortar and pestle (Spot Test Plate -Thomas Scientific) after 400 (l buffer solution is added (50 mM Tris-HCl pH 8.0, 25mM EDTA, 300mM NaCl and 1% SDS). Grind the sample until the buffer solution is green. Add 400(l of buffer solution and mix well. Transfer 400 (l lysate to a test tube with the original leaf sample. Lysate triggers a protein splitting reaction by adding 400(l chloroform. The floating object (supernatant) is transferred into a new test tube (1.5 ml) and DNA is agglomerated using ethanol alcohol. The DNA sample was dried by wind and agglutinated in 50(l of buffer TE (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0). Use aliquot 1(l for PCR analysis. DNA samples are stored in a refrigerator -20°C deep for use. 28 primer pairs were used for evaluation according to table 1.

PCR Tests PCR amplification was performed in 10mM Tris-HCL (pH 8), 50mM KCl, 1.5mM MgCl2.1 units of Taq HATRI, 4 nmol dNTP, 10pmol primer and 50ng genomic DNA. PCR cycles: double wire separation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 60 seconds, 36°C for 60 seconds and 72°C for 120 seconds. The final cord extension is 72°C for 5 minutes. Add 13(l buffer solution (98% formamide, 10mm EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol) after PCR. Polymorphism in the PCR product was detected by ethidium bromide dye after electrophoresis above 5% agarose gel. Data analysis: based on NTSYS-pc software version 2.1.

PCR reaction products with SSR marker

To detect polymorphisms of 20 varieties of stick beans, 44 primers were used in a PCR reaction on DNA genome obtained from leaf samples of 20 varieties of stick beans. The amplification product generated from these primers was observed on 1.5% agarose gel. Observations showed that 28 primers amplified over 100% of the samples, while 20 primers did not amplify any band. The number of DNA fragments produced in a reaction is noted on the table (table 1). Based on the differences between alleles shown in the bandages on the gel, it is possible to determine the differences between the breeds genetically.

After the PCR reaction is complete, 2.5 µl of 6X loading dye solution (MB1 Ferment Inc., Maryland, USA) is added to the PCR product and 1.5% agarose gel electrophoresis (m/v) with 1X TAE buffer solution, then the gel is stained with ethidium bromide and the results are recorded using the system (Syngene, Cambridge, UK). Repeat the electrophoresis process 3 times to observe the ice locations accurately as well as the concentration of each plant with each primer.

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Table 1: Molecular markers from SSR used in bean experiment and polymorphic index and PIC diversity index

No.	Primers	sequencing	replication	No of band	polymorphisum	Retio	PIC	reference
1	BM 200	F-TGTAAACGACGGCCAGTATGCGGTTGGGAAGCCTCATACAG R-ATCTTCGACCCACCTTGCT	(TCT) 10	3	4	68.59	0.32	Rauscher et al.,2013
2	BMd45	F-TGTAAACGACGGCCAGTATGCGGTTGGGAAGC R-CTCATACAG ATCTTCGACCCACCTTGCT	$(AG)_5$	3	9	67.687	0.36	Rauscher et al.,2013
3	PV-ag003	F-TGTAAACGACGGCCAGTATGCTCACGTACGAGT R-TGAATCTCAGGATGGTGTCGGAGAGGTTAAGGTTG	$(AG)_8$	3	8	70.5	0.39	Rauscher et al.,2013
4	BMd10	F-TGTAAACGACGGCCAGTATGCG R-CTCACGTACGAGTTGAATCTCAG	$(GA)_8$	3	9	75.93	0.41	Rauscher et al.,2013
5	BM156	F-TGTAAACGACGGCCAGTATGCCTTGTTCCACCTCCCATCATAGC R-ATCTGAGAGCAGCGACATGGTAG	$(CT)_{32}$	3	7	70.88	0.39	Rauscher et al.,2013
6	GATS91	F-TGTAAACGACGGCCAGTATGCGAGTGCGGAAGCGAGTAGAG R-TCCGTGTTCCTCTGTCTGTG	$(GA)_{17}$	2	12	76.40	0.42	Rauscher et al.,2013
7	BMd47	F-TGTAAACGACGGCCAGTATGCACCTGGTCCCTCAAACCAAT R- CAATGGAGCACCAAAGATCA	$(AT)_5$	10	11	80.99	0.51	Rauscher et al.,2013
8	BMd17	F-TGTAAACGACGGCCAGTATGCGTTAGATCCCGCCCAATAGTC R-AGATAGGAAGGGCGTGGTTT	$(CGCCAC)_6$	3	6	67.67	0.35	Rauscher et al.,2013
9	PVBR107	F-TGTAAACGACGGCCAGTATGCCCCCTTTCTCACCACTTCAG R-ACCAAAAACGGTGCTCAAAC	$(CT)_{16}$ $(GT)_4$	2	7	66.45	0.39	Rauscher et al.,2013
10	BM175	F-TGTAAACGACGGCCAGTATGCCAACAGTTAAAGGTC R-GTCAAATT	$(AT)_5$ $(GA)_{19}$	2	8	85.00	0.44	Rauscher et al.,2013

		CACTCTTAGCATCAACTGGA						
11	J01263	F-ATGCATGTTCCAACCACCTTCTC R-GGAGTGGAACCCTTGCTCTCATC	$(ATCC)_3$ $(AG)_2$	3	2	72.8	0.42	Yu et al.,2000
12	J04555	F-AGGGTGTTTCACTATTGTCACTGC R-TTCATGGATGGTGGAGGAACAG	$(CTT)_3(T)_3$	2	10	75.2	0.38	Yu et al.,2000
13	K03288	F- TGCCACCACAGCTTTCTCCTC R-TATGAGAGAAGCGGTTGGCACG	$(ATGC)_4$	2	2	43.39	0.35	Yu et al.,2000
14	K03289	F-AGCTTTCACACTATGACACCACTGG R-TGCGACATGAGAGAAAGACACGG	$(ATGC)_4$	2	5	65.46	0.39	Yu et al.,2000
15	M18093	F-CCAGCTACCATCTCCTCCATCG R-TAGTGGTGGAGGTGGAGATTT	$(CCA)_6$	2	3	59.00	0.38	Yu et al.,2000
16	M18094	F-TAATTTCTCTCTTCCCATCCCAAAC R-GTAGTAATAAGGAGGAGGCGGTGAG	$(ATCT)_3$	7	15	55.74	0.35	Yu et al.,2000
17	SS715647275	F-ATCTGAGAGCAGCGACATGGTAG R-TATACACACGAACTTTGCATTCCG	$(CT)_8$	12	17	79.25	0.48	
18	SS715649259	F-ACATGCAAGTTCACACGGTCCTC R-ACCTAGAGCCTAATCCTTCTGCGT	$(TCTTTC)_6$	12	17	82.4	0.55	
19	M75856	F-CAATCCTCTCTCTCTCATTTCCAATC R-GACCTTGAAGTCGGTGTCGTTT	$(GA)_{11}$	4	19	54.46	0.32	Yu et al.,2000
20	U10419	F-TGGAGCCATCTGTCTCTTACCCAC R-GAGCACGAGTCACGTTTGCAAC	$(AAAT)_3$	2	6	55.4	0.38	Yu et al.,2000
21	U18349	F- CTGAAGCCCGAATCTTGCGA R- CGCGAGAGGTGAACGAAAGC	$(GGC)_5$	2	9	66.24	0.35	Yu et al.,2000
22	U18791	F- GGGAGGGTAGGGAAGCAGTG R- GCGAACCACGTTCATGAATGA	$(TA)_{22}$	8	7	57.68	0.33	Yu et al.,2000
23	U28645	F-GCAAGAGAACACTGAAGAGGATCG R-GACATTACTCATTTCATCATCTAC	$(CCA)_5$	3	15	74.51	0.41	Yu et al.,2000

		TACACG						
24	U34754	F-GTTTCTTCCTTATGGTTAGGTTGTTTG R-TCACGTTATCACCAGCATCGTAGTA	$(AT)_8$	4	17	68.45	0.36	Yu et al.,2000
25	U54703	F- CGAGGAGGAAGGAGAAGACGG R-GAGGGTTATCACAAGGAAGACACG	$(TTA)_4$	4	12	61.23	0.35	Yu et al.,2000
26	U77935	F- CGTTAGATCCCGCCCAATAGT R- CCGTCCAGGAAGAGCGAGC	$(GCCACC)_5$	2	6	66.57	0.36	Yu et al.,2000
27	X04001	F-TCACGTACGAGTTGAATCTCAGGAT R-GGTGTCGGAGAGGTTAAGGTTG	$(AG)_8$	2	6	58.56	0.33	Yu et al.,2000
28	X04660	F- TTGATGACGTGGATGCATTGC R-AAAGGGCTAGGGAGAGTAAGTTGG	$(AG)_8$	6	8	74.25	0.49	Yu et al.,2000
Total				**111**	**257**	**65.65**	**0.384**	

Statistical analysis The SSR tape will be displayed based on molecular mass and in kilo base (kb) based on the scale as a marker. The data is processed using MS Excel software to calculate the polymorphic tapes of each individual primer, polymorphic average and polymorphic scale. Analysis of potential information of molecular markers and genetic diversity in assessed genotypes including the number of alleles on the desired locus–Aep according to (Weir BS et al 1996), Shannon diversity index- (Martynov SP et al. 2003) genetic diversity/diversity index- Hep, marker index (MI) and polymorphic content - PIC is calculated for each primer on a plant based on the allele frequency on the locus of each plant.

Cluster Analysis

SSR will be encrypted in binaries 0 and 1. On horizontal electrophoresis gels, the sample with the tape is recorded as 1, the sample without the tape is recorded as 0. Based on the tape results, the matrix represents the genetic correlation for all pairs from Euclidean Distance and is used to construct the family tree schema according to the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The data will be analyzed using IBM SPSS Statistics 20 software. The data from the SSR marker will be processed together

III. RESULTS AND DISCUSSION

The variance analysis (ANOVA) showed a statistically significant phenotype ($p < 0.05$) for all characteristics of the variety, date of first flowering, plant height, flowers per plant, fruit weight (Table 2). Based on morphological parameters, the average value of characteristics related to flowering date, height of plant, number of fruits on plant with 20 varieties of beans is analyzed on table 2. The bean-like genotype has the very early appearance of 50% of flowering flowers (20 days after seeding) for Alubia beans, while the bean variety imported from the United States recorded the longest flowering period (40 days after seeding) of 50% female flowering. The number of days of flowering ranged to 24 days for Alubia (23.93) and the United States (40.66), respectively, while 13 days were found to be the average number of days to appear. The number of flower varieties also varies HaLan (78.76 flowers) varieties with the number of l cotton t ...The variety with the lowest L number of flower is the Cong

Ty bean variety (36.39 flower per string).The color of seed flowers is also rich white, black and brown .

Table 2. Evaluation of physiological characteristics of 20 varieties of Bean sticks in the Winter-Spring crop 2021 planted in Can Tho

no	lines	Flowering (days)	Hight plants (cm)	Color flower	Color seed	Second branch	No flower
1	Alubia	23,92d	193,19b	white	white	1,57ab	44,54b
2	Osu544C	35,91b	221,04ab	white	white	3,80c	69,43ab
3	Hà Lan	30,87c	300,40a	white	Large white	4,33efg	78,76ab
4	Pháp	32,24ab	188,47b	white	Small white	3,58def	56,76ab
5	Brasil	36,89ab	194,76ab	Purple	black	5,90de	77,51ab
6	Áo	39,58a	184,75b	red	White and spot red	2,78ab	75,02ab
7	HoaKỳ	40,66a	198,68ab	Gray	gray	7,03fg	40,05ab
8	Anh	33,56ab	196,85ab	Purple	gray	5,90h	67,84ab
9	Ấn Độ	39,91a	198,81ab	Whie	gray	7,06fg	68,92ab
10	Bhatle	37,90a	203,41ab	red	black	4,20bc	55,32ab
11	Chiese Long	31,30c	190,71ab	red	Black	7,60def	66,12ab
12	WhiteOP	30,43c	172,15b	White	White	6,85g	68,31ab
13	DHundi	37,66a	194,35ab	Purple	There are black spots	8,10d	75,95ab
14	LB39	36,51ab	192,25ab	Whte	White	4,11a	66,38ab
15	Chaumese	41,11a	189,681b	red	black	7,16fg	68,99ab
16	Thái Lan	37,50a	203,47ab	white	white	5,50bc	66,32a
17	Bolu (HQ)	31,20c	190,71ab	white	white	7,65def	56,12ab
18	Philippine	30,53c	222,25a	white	white	6,45g	48,13ab
19	Đài Loan	37,55a	197,36ab	white	white	8,18d	55,95a
20	CôngTy	36,69ab	194,23ab	white	white	4,47a	36,39ab
	CV (%)	14,01	8,63			2,05	12,27

Note: Numbers that follow the same character have no statistical significance difference at 5%. - **Yield and yield composition recorded**: the number of seeds/fruits ranges from 5 to 11 seeds. The Bhatle bean-like genotype exhibits a maximum average weight (1.87 g), The highest fruit length is the Osu544C and Brasil bean variety long (17.9-17.44 cm) repectity . The width ranges from 1.47 – 2.23cm. Productivity ranges from 0.70 to 1.8kg/wire. The tallest breed is still the Bhatle. Through the analysis of agronomic traits, yield and yield composition of 20 varieties, we show that Bhatle variety followed by White OP is somewhat superior to other varieties, Dai Loan bean variety is the lowest yielding variety (Table 3).

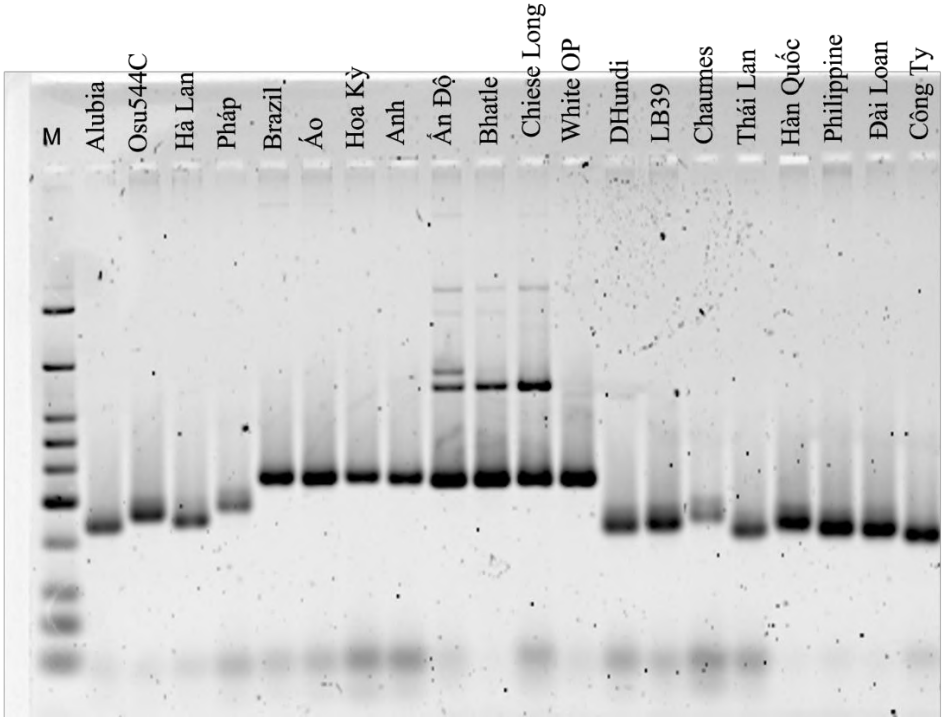
Table 3: Yield and yield composition of 20 varieties of bean melons in the Winter Spring crop 2021 in Can Tho

no	Giống	Numbers seeds/ pod	Length fruits (cm)	wide (cm)	Number pod / plant	Yield/plant (kg)	Yield/ ton/ ha
1	Alubia	8d	14.23d	2.23a	80	0.79h	8.47e
2	Osu544C	9c	17.90b	1.47b	58	1.51d	11.53a

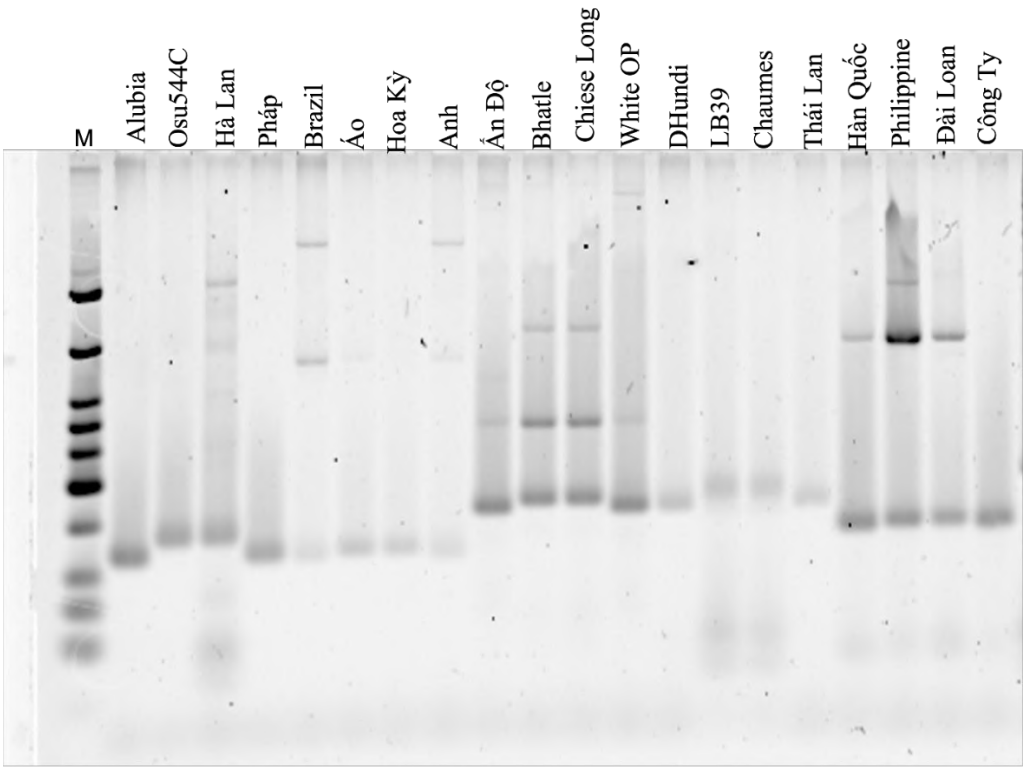
3	Hà Lan	8d	16.10bc	2.56a	37	1.35e	9,23d
4	Pháp	8d	15.82c	1.41b	42	0.70h	8.66e
5	Brasil	8d	17.44ab	1.09c	42	0.77h	7.97f
6	Áo	5g	14.17d	1.97ab	40	0 .83h	9.13d
7	Hoa Kỳ	7e	16.33bc	1.92ab	50	1.18g	9.90d
8	Anh	4h	14.46d	1,79ab	82	1.02g	9.39d
9	Ấn Độ	9c	16.96abc	1.88ab	32	1.56c	11.86b
10	Bhatle	11a	14.40d	1.07c	41	1.86a	12.94a
11	Chiese Long	8d	13.28de	1.49b	60	1,04g	7.04f
12	White OP	8d	12.24e	1.79b	59	1.78b	11.33b
13	DHundi	8d	13.50de	1.87ab	41	1.10g	10.52c
14	LB39	10b	13.07de	1.06c	40	1.64c	11.38b
15	Chaumese	6f	15.34c	1.12c	39	1.69c	8.55 e
16	Thai Lan	5g	11.6d	1.5b	41	0.85h	7.14f
17	HanQuoc	6f	15.20c	1.65b	37	1.17g	9.15d
18	Philippine	6f	13.67de	1.32b	72	0.8h	8.14e
19	DaiLoan	7e	16.12bc	1.41b	35	1.13g	5.85g
20	Công Ty	5g	16.11bc	1.6b	33	0.94h	7.36f
	C%	**4.33**	**5.45**	**4.11**	**2.16**	**1.12**	**11.28**

Note: Numbers that follow the same character have no statistical significance difference at 5%.

PCR reaction products with SSR marker method Use 24 SSR primers to amplify stick bean plants, in which all eSSR products are recorded for polymorphism over 20 bean stick varieties (P = 100%). For SS715649259 DNA amplification for the product reaches 100% The product amplifies 12 alleles with molecular sizes ranging from 180bp to 400bp. Alubia (210 bp), OSU 544C (220bp); Ha Lan(210bp), Phap(250bp), Brazil (300bp), Austria (300bp), Hoa Ky(300bp), Anh (300bp), India (300;320,350,400 bp); Bhatle (300;350,400), Chiese Long (300;350,400); White OP (300bp); Dhundi (210bp); LB39 (210bp), Chaurmes (220bp); Thailand (200bp), South HanQuoc(210bp), Philippines (200bp), Đai Loan(200bp), Cong Ty(180bp) (Figure 1A). For SS 715647275 DNA amplification for the product reaches 100% The product amplifies 12 alleles with molecular sizes ranging from 250bp to 320bp. Alubia (210 bp), OSU 544C (220bp); Netherlands (220;400bp), France (210bp), Brazil (210,350,400bp), Austria (2050bp) United States (205 bp) Anh (210,450 ...) Ấn Độ (250; 330,360bp); Bhatle(260;300,380bp), Chiese Long (260;300,380bp); White OP(250,300bp) Dhundi(250bp); LB39(270bp), Chaurmes(270bp); Thai Lan (206bp), Han Quoc (240bp,380), Philippine (240bp,380), Đài Loan (240,380bp), Công Ty (240bp) (figure 1B).



(A)



(B)

Fig.1: PCR products of SS715649259(A) and SS715647275(B) on 20 different bean varieties separated on polyacrylamide gel with silver nitrate dyeing

Genetic diversity assessment of 20 stick bean varieties using NTSYSpc.2.1 software The results of grouping 20 bean varieties based on phenotypic data and calculation results using NTSYSpc.2.1 software are

presented as subgroup trees combining phenotype and genotype. Figure 2. Subgroup trees show the relationship of genetic distance between varieties based on the correlation between varieties and their contribution to the diversity index of molecular markers. The results of genetic grouping based on genotype show that: at a genetic distance of about 0.74 breeds are divided into 4 very pronounced groups: Groups A, B, C, and D. Thus, with 28 primers, 20 varieties are classified into 4 main groups, in which the correlation between varieties ranges from 0.39 – 1.0, indicating that the breeds have high genetic diversity. The degree of similarity between the varieties was as low as 0.39% and some varieties in group A had the highest degree of similarity of nearly 100%. Genetic differences between breeds in group A are higher than in breeds in group C, the coefficient of similarity.Genetic differences between breeds in group A are higher than in breeds in group C, the similarity coefficient between group A and group B compared to group C is 0.41 and breeds in group B can be considered intermediate breeds between group A and group C. This means that if the breeds in groups A and C are crossbred with group C B can produce more individuals with many desirable traits because the longer the genetic distance, the higher the likelihood of hybrid superiority (Bui Chi Buu et al. 2003).

Genetic subtypes are based on their ability to combine with primers, at similarities 0 to 0.41 varieties are divided into two main groups A, B, C, D:

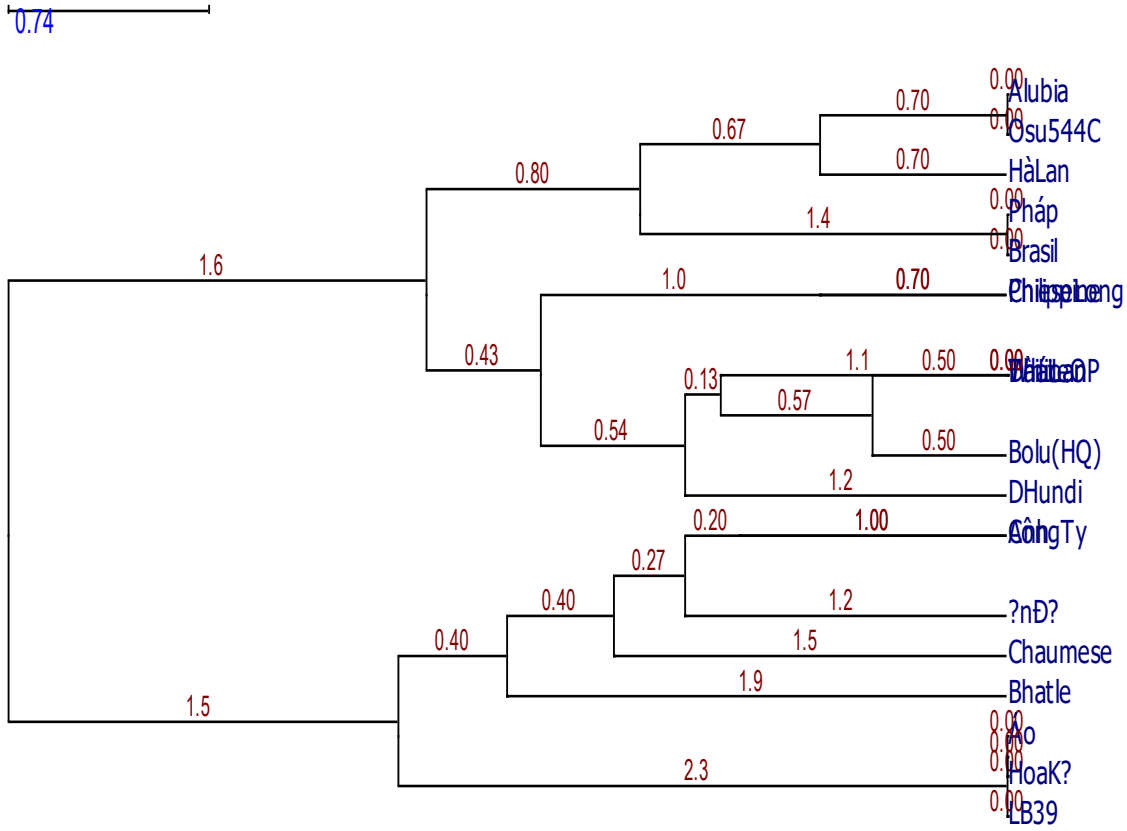


Fig.2. The genealogy schema shows the genetic correlation between 20 bean varieties based on the Jaccard Homology Index using the SSR marker (UPGMA). Four groups when considering a similarity coefficient less than 0.74

- Group A: Group A has a yearly similarity in the range of 0.00-0.40, including 3 varieties: LB39, stick beans imported from the Hoa Ky and Ao (with indicator SS715649259) with a molecular size of 300bp. Group B: there are 5 varieties: Bhatle, Chaumese, DaiLoan, An Do, cong ty . Group C: is divided into three groups C1, C2 and C3 at a similarity coefficient of 0.50. Group C1 has 1 variety of Dhundi. Group C2 includes the following

varieties: 2 varieties: Bolu (HQ) and Thai. Group C3: White OP, UK Group D: includes 2 varieties: Chiese Long and Philippne. Group E: includes 5 varieties: stick beans from Brazil, Phap, HaLan, OSU 544 C and Alubia. Group E1 includes 2 varieties: Brazilian, Phap. Group E2: includes three varieties: Dutch, OSU 544 C and Alubia.

IV. DISCUSSION

Phenotypic analysis noted bean varieties with different meanings of different fruit forms. Among the 28 primers (Fig. 1) show the lines of the trees in a uniform way. SSR primers with a total of 257 band lines have 111 alleles that exhibit polymorphism. Polymorphism is expressed through markers of varieties valued from 28.57% (EC45). The average number of DNA fragments amplified by the SSR marker in this study ranged from 2 to 12 alleles (size 180-400bp). With 28 markers, there is a high variability of DNA fragments produced from the SSR marker, which can be attributed to differences in attachment sites across the entire set of alleles of different bean varieties. The effectiveness of the primers used in SSR is to determine the quantity by estimating based on differences in genetic parameters (table 1). In the analysis of genetic diversity divided into important components: allele frequency and polymorphism in the group. To assess genetic diversity between different breeds/lineages of geographical origin in the many traits are abundant and find genetic gaps to establish plausibility for later hybrid material. For SSR for polymorphic locus ratio:

At a significance level of 1%, the percentage of polymorphic loci among seed groups ranged from 59% to 100%. Polymorphism manifests itself most importantly in different groups of breeds. Average number of alleles per locus: In general, in most loci there are two or three alleles, except for the alele on molecules SS715649259 and SS, which 715647275 have 12 alleles. The genetic diversity index is (H) H = 0.384. However, the Shannon Diversity Index, which evaluates only on primers in different melon varieties, indicates the similarity of narrow genetics of the plants studied. When comparing PIC values, recorded a wide range of PIC values in this topic with 20 varieties ranging from 0.32 (BM 200) to 0.55 (SS 715647275) with an average of 0.384. Genetic diversity (Hep) is polymorphic, demonstrating the effectiveness of the eSSR loci information in this study also noted polymorphisms with high on primers such as M75856.

In this study, Hep showed remarkable homogeneity across each SSR primer and ranged from 0.85% (BM175) Furthermore, Hep showed a positive and significant association (r = 0.914, P < 0.001) with allele/locus influence (11.32 to 11.95). The marker's parameter was calculated to detect the characteristics of each individual using a separate primer to identify polymorphic loci on different melon varieties. Based on the number and frequency of scoring of DNA fragments, polymorphism rates, and other efficacy parameters after combining, it appears that these SSR instructions suggest that polymorphisms are best effective markers and can be used to screen molecules in later high-yielding melon gene pools. The electrophoresis tape analyzed by UPGMA method (figure 1) shows the genotypic grouping process into 4 main groups. There is a strong association between the genotypes that have been documented. Such genetic correlation is very significant, it provides for breeds in the crossing program.

All traits (except dates of appearance) reflect significant genotyping, indicating the existence of genotypic variation useful for breeding purposes (Table 2). The genotypes observed in this experiment were found to be consistent with previous reports (Okii etal.,2018). In this study, all traits were found to be hereditary except for dates of occurrence, root length, and secondary branches on a scale of (Robinson et al.,1966) (Table 2). The degree of heredity is mainly governed by the degree of genetic variation, while higher heredity leads to a lower environment on a particular trait (Phuke et al.,2017). Variance analysis (ANOVA) for most of the traits studied reflected that genotypic variance was significant within as well as across the environment, indicating a higher degree of their heredity. The findings of this study are consistent with previous studies (Okii et al.,2018, Wondimu et al., 2017) stating that in the days leading up to maturity, fruit per tree, number of seeds per tree, and weight of 100 seeds are less affected by environmental forces and are highly heritable traits over many years/places.

The wide range of phenotypic values obtained for the 12 traits reflects the occurrence of important variation for different agromorphological traits in the study of bean sprouts of many countries, which was found to be consistent with previous studies (Rana et al., 2015, De La Fuente et al.,2013). Seed-related characteristics are considered important for stick beans and are considered a major determinant of the commercial acceptability of commercial varieties (Rana et al.,2015). Fruit / string weight is an important characteristic, which has a positive and significant impact on stick bean yield. The average weight of 100 grains (42.2 g) resulted in this study which was significantly higher than previous studies (Bozo ̆glu et al.,2011, Yeken et al.,2019, Yeken, et al.,2018). The higher average seed weight noted in this study may be due to the inclusion of large numbers of joinings that have larger seed sizes than in previous studies. Voysest et

al.,1983 claim that p...Given that the weight of 100 grains of regular beans can vary between < 15 to > 90 g per 100 grams

In addition to seed color, growth habits also reflect variations in the height of the plant. Climatic conditions and human preferences can play an important role in the distribution of bean in a particular area (Rana et al.,2015). In this study, climatic conditions noted French, Austrian varieties of determined growth with early maturity. The length and width of the left remain in the shell. Similar findings were reported by Balkaya and Ergün (Balkaya et al.2008) on the use of genotypes of variable-length creeping pea varieties (Table 2) Selecting the gene pool that performs best and is stable in multiple environments per year is also one of the objectives of this study.

V. CONCLUSTION

-Morphological parameters, average values of characteristics related to flowering date, string tall, number of leaves, number of cotton of genotypes on 20 varieties of beans are analyzed. The Alubia genotype has a very early appearance of 50% of flowering female flowers (23.92 days after sowing), while the Chaumese variety recorded the longest flowering period (41.11 days after sowing).

- Analysis on SSR markers: Through the SSR marker data with 28 primers used, 20 varieties are classified into 4 main groups. The SSR marker has proven to be a powerful tool for determining how to genetically diversify across 20 varieties of beans to better manage the bean gene bank also to promote the use of imported varieties in future breeding programs. In the subgroup of the SSR on 28 molecular markers are noted with 4 distinct groups. A grouping map that coordinates two molecular marker methods is also established with four different groups. Relying on molecular markers to be able to indirectly assess the presence or absence of selected genes thanks to markers without environmental influences.

- Diversity index analyzed by high SSR method (H = 0.384) while the diversity index of beans. The results presented here are the first steps towards a better understanding of bean varieties introduced from countries and may help breeding program in future.

ACKNOWLEDGMENTS

The author sincerely thanks the project. Thank you to Can Tho City Department of Science and Technology for funding and data discussion. Thank you to the Mekong Delta High-tech Agricultural Research Institute for funding to facilitate the laboratory to implement this project.

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