Jurnal Ilmu Pertanian Indonesia (JIPI), April 2025
(Indonesian Journal of Agricultural Sciences)
ISSN 0853-4217 EISSN 2443-3462

Vol. 30 (2): 293–304
http://journal.ipb.ac.id/index.php/JIPI
DOI: 10.18343/jipi.30.2.293

Genetic Diversity and DNA Barcoding Construction of Tropical Soybean Advanced Lines Based on SSR Markers

Kunto Wibisono[1,2], Rosliana Purwaning Dyah[2], Ratna Utari[2], Suparjo[2], Umar[2], Habib Rijzaani[2], Lukman Hakim[2], Ace Suhendar[2], Oky Dwi Purwanto[2], Dani Satyawan[3], Witjaksono[3], Mastur[4], Puji Lestari[5], I Made Tasma[2]*

(Received March 2024/Accepted January 2025)

ABSTRACT

Soybean cultivation in tropical regions, such as Indonesia, is often constrained by photoperiod sensitivity, resulting in low yield. Using long juvenile traits in short photoperiod tropical areas resulted in lines with late flowering time and high yield. Genetic diversity analysis of soybean lines using molecular markers is a critical step for breeding high-yielding soybean lines. This study aimed to analyze genetic diversity and construct DNA barcodes for 44 tropical soybean advanced lines based on 17 SSR markers. Genetic materials used were the high-yielding F5 soybean lines developed for their adaptation to short day-length of low latitude tropical regions. SSR markers used were those that distributed well across the soybean genome and proven their usefulness for soybean genetic diversity analyses. Results showed that the SSR demonstrated distinctive polymorphism among the 44 lines. A total of 377 alleles were detected with an average of 22.8 alleles per SSR locus. Polymorphism information content (PIC) values varied from 0.77 to 0.96 with an average of 0.90. Phylogenetic analysis showed that the 44 soybean genotypes were divided into 2 main clusters. Five markers, i.e., *satt009*, *satt646*, *satt147*, *satt431*, and *satt191*, with a polymorphism information content value of ≥0.94, were found to be informative and suitable for DNA barcode construction. Each of the 44 lines was assigned with specific barcodes. The barcodes constructed from this study should be useful for DNA fingerprint as well as protection purposes of the specific superior soybean lines analyzed in this study.

Keywords: DNA fingerprint, molecular markers, photoperiod, plant breeding, soybean

INTRODUCTION

Indonesia's domestic soybean commodity demands in 2021 are still fulfilled by imports, particularly from the United States (2,152,633 tons), Canada (232,009 tons), Argentina (89,951 tons), and Brazil (9,238 tons) (BPS 2021). One of the reasons contributing to low soybean yield is an incompatible adaptation between the soybean genotype and the agricultural area (Spehar 1995; Hartwig and Kiihl 1979). Until the late 1960s, soybean agriculture was limited to locations above 22° latitude due to photoperiod barriers (Carpentieri-Pípolo *et al*. 2002; Gupta *et al*. 2021).

Brazil's soybean tropicalization program (dos Santos Silva *et al*. 2017) has successfully addressed photoperiod limitations in the tropics (<20° latitude) by introducing a soybean genotype with a long juvenile trait (*lj*) from the southern US. Indonesia began a breeding program with *lj* trait in 2017 and has developed numerous outstanding *lj* lines (Tasma *et al*. 2018). The created lines are not only very productive, but they also exhibit huge seed size and resistance to pod-shattering trait. Other lines have lengthy root trait that may indicate drought tolerance. Some of the parents used have high isoflavone content, the lines created may have as well.

Genetic diversity analysis is critical in soybean breeding programs because it enables the discovery and assessment of differences across different soybean lines (Dong *et al*. 2004). According to Liu *et al*. (2020) and Manjarrez-Sandoval *et al.* (1997), crossing parents with a long genetic distance results in superior offspring. This information assists breeders in making informed decisions when selecting suitable parents for future breeding programs, to develop better soybean varieties with desirable traits such as high productivity (Lu *et al*. 2017), disease resistance (Presello *et al.*

[1] Graduate School, IPB University, IPB Campus Darmaga, Bogor 16680, Indonesia
[2] Food Crops Research Center, Agricultural and Food Research Organization, National Research and Innovation Agency (BRIN), Bogor 16911, Indonesia
[3] Genetic Engineering Research Center, Biological and Environmental Research Organization, National Research and Innovation Agency (BRIN), Bogor 16911, Indonesia
[4] Center for Standard Testing of Biotechnology Instruments and Agricultural Genetic Resources, Ministry of Agriculture, Jl. Tentara Pelajar, Bogor 16111, Indonesia
[5] Agriculture and Food Research Organization, National Research and Innovation Agency (BRIN), Bogor 16911, Indonesia
* Corresponding Author: E-mail: i.made.tasma@brin.go.id

2005), and adaptation to specific environmental conditions (Liu *et al*. 2023).

In addition, maintaining the genetic identity of a plant variety is essential in protecting it from unauthorized use (Valentini *et al*. 2009). Genetic identity can be determined through DNA barcoding using molecular markers that are accurate and not affected by environmental factors (Li *et al*. 2015). Therefore, the evaluation of genetic diversity and the creation of genetic identity using DNA barcoding is important to be carried out on superior lines of tropical soybeans that have high productivity developed by marking-assisted methods.

Molecular markers, such as Simple Sequence Repeats (SSR), are widely used in soybean research for genetic diversity analysis, phylogenetic studies, fingerprinting analysis, genetic mapping, and Quantitative Trait Loci (QTL) of important traits (Tasma *et al*. 2001; Tasma *et al*. 2003), as well as marker-assisted selection. SSR markers have codominant qualities, are very reproducible, are distributed throughout the genome, can detect high polymorphism by polymerase chain reaction (PCR) amplification, and are simple to amplify using PCR techniques (Singh *et al*. 2018; Amiteye 2021). This study aimed to analyze genetic diversity and construct DNA barcodes for 44 tropical soybean advanced lines based on 17 SSR markers.

METHODS

Genetic Materials and SSR Markers

The genetic material used were 44 tropical soybean advanced lines (F5) with high productivity and harvest times ranging from 95 to 115 days after planting (dap). These lines have high seed sizes and are resistant to pod-shattering. These lines were created using the mark-assisted pedigree and mark-assisted backcross methods, which have been developed since 2017 (Tasma *et al*. 2018). These tropical soybean lines were created by crossing female parent (Grobogan) and five soybean genotypes with *lj* trait brought from the United States as male parents (Table 1). These outstanding tropical soybean lines were planted at the Cikeumeuh Experimental Station in Bogor, West Java, Indonesia. From January to May 2023, SSR markers were analyzed at the Genomics Laboratory of the National Research and Innovation Agency (BRIN) in Cibinong, West Java. This study used 17 SSR markers (Table 2). The selection of 17 SSR markers in soybeans was based on high levels of polymorphism, even distribution across the genome, demonstrated stability and reproducibility, and has been widely used in prior studies (Lestari *et al*. 2021; Asadi *et al*. 2020).

Genomic DNA Isolation, Quantitative and Qualitative Analysis

Table 1 Characteristics of 44 tropical soybean advanced lines used in this study

Genotypes	Genetic background[a]	Gene *lj*	Seed size[b]	PSR (*pdh1*)[c]	Genotypes	Genetic background[a]	Gene *lj*	Seed size[b]	PSR (*pdh1*)[c]
UD-9	G × M	Yes	Medium	No	UD-12	G × M	Yes	Medium	No
UD-24	G × P	Yes	Medium	Yes	UD-13	G × V	Yes	Medium	No
UD-26	G × P	Yes	Large	Yes	UD-32	[G × (G × P)]	No	Medium	No
UD-27	G × P	Yes	Large	No	UD-33	[G × (G × P)]	No	Large	No
UD-28	G × P	Yes	Large	No	UD-36	[G × (G × P)]	No	Medium	No
UD-38	[G × (G × P)]	No	Medium	No	UD-37	[G × (G × P)]	No	Large	No
UD-40	[G × (G × P)]	No	Large	No	UD-39	[G × (G × P)]	No	Large	No
UD-44	[G × (G × P)]	No	Large	No	UD-42	[G × (G × P)]	No	Large	No
UD-48	G × M	Yes	Large	No	UD-62	G × M	Yes	Large	Yes
UD-64	G × M	Yes	Medium	Yes	UD-63	G × M	Yes	Medium	Yes
UD-67	G × M	Yes	Medium	No	UD-70	G × V	Yes	Large	Yes
UD-68	G × M	Yes	Large	No	UD-71	G × V	Yes	Medium	Yes
UD-73	G × V	Yes	Medium	Yes	UD-72	G × V	Yes	Medium	No
UD-74	G × V	Yes	Large	Yes	UD-81	G × V	Yes	Large	No
UD-76	G × V	Yes	Large	No	UD-86	G × Gy	Yes	Large	No
UD-80	G × V	Yes	Large	Yes	UD-89	G × V	Yes	Medium	No
UD-93	[G × (G × P)]	No	Large	No	UD-90	G × H	Yes	Medium	No
UD-96	G × M	Yes	Medium	No	UD-104	[G × (G × P)]	Yes	Medium	No
UD-99	G × Gy	Yes	Medium	Yes	UD-106	[G × (G × P)]	No	Large	No
UD-105	[G × (G × P)]	Yes	Large	No	UD-115	G × M	Yes	Large	No
UD-114	G × M	Yes	Medium	No	UD-117	G × V	Yes	Medium	Yes
UD-116	G × M	Yes	Large	No	UD-118	G × V	Yes	Medium	Yes

Remaks: [a]G = Grobogan; M = Melrose; V = Vernal; Gy = Glycine H; P = Paranagoiana; H = Hinson lj. [b]Large seeds if the weight of 100 seeds ≥ 14 g, medium 11–13 g, small 6–10 g. [c]PSR = pod-shattering resistance based on *pdh1* gene.

Table 2 Characteristics of 17 SSR markers used in this study (Cregan *et al*. 1999)

SSR[a] Marker	Chromosome	Repetition type	Primer sequence[b] (5' → 3')	PCR[c] Product size (bp)
satt002	17	(TA)5tgtacgattt aaaaataaaata(AT)5	F: TGTGGGTAAAATAGATAAAAAT R: TCATTTTGAATCGTTGAA	126
satt009	3	(ATT)14	F: CCAACTTGAAATTACTAGAGAAA R: CTTACTAGCGTATTAACCCTT	162
satt030	13	(ATA)21	F: AAAAAGTGAACCAAGCC R: TCTTAAATCTTATGTTGATGC	164
satt038	18	(ATT)17	F: GGGAATCTTTTTTTCTTTCTATTAAGTT R: GGGCATTGAAATGGTTTTAGTCA	176
satt045	15	(AAT)18	F: TGGTTTCTACTTTCTATAATTATTT R: ATGCCTCTCCCTCCT	139
satt063	14	(TAA)20	F: AAATGATTAACAATGTTTATGAT R: ACTTGCATCAGTTAATAACAA	144
satt114	13	(AAT)17	F: GGGTTATCCTCCCCAATA R: ATATGGGATGATAAGGTGAA	108
satt147	1	(ATA)15	F: CCATCCCTTCCTCCAAATAGAT R: CTTCCACACCCTAGTTTAGTGACAA	172
satt191	18	(TAT)19	F: CTTCCACACCCTAGTTTAGTGACAA R: GGGAGTTGGTGTTTTCTTGTG	226
satt194	4	(ATT)4gag taaatag(TA)5	F: GGGCCCAACTGATATTTAATTGTAA R: GCGCTTTGTGTTCCGATTTTGAT	246
satt197	11	(ATT)20	F: CACTGCTTTTTCCCCTCTCT R: AAGATACCCCCAACATTATTTGTAA	173
satt294	4	(TAT)23	F: GCGGGTCAAATGCAAATTATTTTT R: GCGCTCAGTGTGAAAGTTGTTTCTAT	287
satt308	7	(TTA)22	F: GCGTTAAGGTTGGCAGGGTGGAAGT R: GCGCAGCTTTATACAAAAATCAACAA	170
satt431	16	(AAT)21	F: GCGTGGCACCCTTGATAAATAA R: GCGCACGAAAGTTTTTCTGTAACA	230
satt463	7	(AAT)13(GAT) 17 (AAT)19	F: TTGGATCTATATTCAAACTTTCAAG R: CTGCAAATTTGATGCACATGTGTCTA	221
satt607	4	(AAT)15	F: GCGGTTTCATCTGCAGTGTATTATTAT R: GCGCCACTTAATTATTTCAGATTAATT	225
satt646	4	(TTA)11	F: GCGGGGTATGAATTAATTAATGTAGAAT R: GCGCCTTCAAAAACTAATGACATATCAT	199

Remaks: [a]SSR: Simple Sequence Repeats. [b]F: Forward; R: Reverse. [c]PCR: Polymerase Chain Reaction; and bp = base pair.

Genomic DNA was isolated from young leaves using a modified Doyle & Doyle (1990) method using 2% (w/v) PVP in the extraction solution. The DNA was diluted with 100 µL TE (Tris 10 mM [pH 8.0], EDTA 1 mM) and 2 µL RNAse 10 mg/mL (Invitrogen, USA) and incubated at 37°C for 1 hour. Next, genomic DNA was electrophoresed on a 1% agarose gel (Sambrook *et al*. 1989), and the DNA bands were detected with a UV Transilluminator (UVP, UK). The concentration and purity of DNA were measured using a Nano Drop 2000 spectrophotometer (Thermo Scientific, USA).

PCR and Electrophoresis Analysis

The PCR reaction was done in a total volume of 10 µL, consisting of 2 µL of template DNA (20 ng), 2 µL of Kapax2G Fast Ready Mix (KAPA Biosystem, USA), 0.5 µL of each forward and reverse primer (10 µM), and 2 µL of sterilized ddH_2O. The PCR cycle conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of DNA denaturation at 94°C for 30 seconds, primary annealing at 55°C for 1 minute, DNA extension at 72°C for 1 minute, post DNA extension at 60°C for 15 minutes, and incubation of the DNA at 10°C for 4 minutes. The PCR product was electrophoresed with an 8% polyacrylamide gel at 90 V. The DNA bands were seen on a UV Transilluminator Gel Doc (Bio Rad, USA) with an ethidium bromide staining technique.

Data Analysis

GelAnalyzer v.2010a software (Lazer and Horvath-Lazar 2010) was used to capture SSR allele band patterns. The recorded data was analyzed using PowerMarker V3.25 software (Liu and Muse 2005) to calculate the polymorphism information content (PIC) value, major allele frequency, allele size range, genetic diversity, and heterozygosity value for each SSR marker. Dendrograms were generated using NTSYS-pc software version 2.1 (Rohlf 2000) and the sequential agglomerative hierarchical and nested (SAHN) - unweighted pair group method with arithmetic (UPGMA).

DNA barcoding profiling consisted of multiple processes, including the selection of SSR marker candidates, the development of a specific identity (ID), and the construction of a barcode for each genotype. The selected SSR mark for ID construction must have a PIC value greater than 0.5 (Botstein *et al.* 1980). Several SSR markers were used in the phylogenetic study (Chung *et al*. 2009). The DNA barcode profile was created using a numeric code by assigning a two-digit specific code obtained from the allele size range based on the GelAnalyzer software's analysis of each selected SSR marker. The allele size of each SSR marker was estimated using the GelAnalyzer software analysis (Lestari *et al.* 2021). Barcodes for each genotype were constructed based on numeric set numbers available barcode website (Barcodes 2021) using an ID.

RESULTS AND DISCUSSION

SSR Marker Analysis

In this study, 17 SSR markers were employed to analyze diversity among genotypes (Figure 1). A total of 377 alleles were found in 44 genotypes, with an average of 22.18 alleles per locus, ranging from 11 to 39 alleles (Table 3). The *satt191* and *satt646* markers possessed 39 numbers of alleles across the genotypes. Uneven recombination and crossing-over events occur throughout genotype formation, as

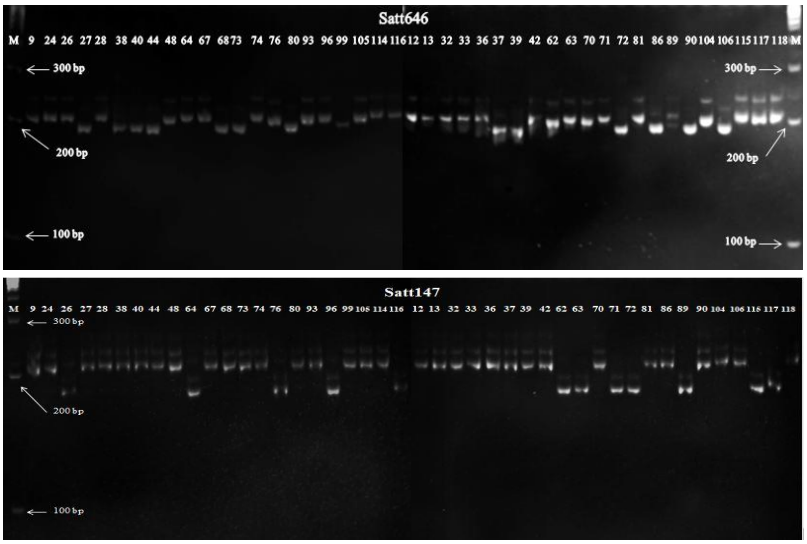

Figure 1 Electropherogram of DNA band pattern produced by *satt646* (a) and *satt147* (b) markers, migrated on 8% polyacrylamide gel. M = 100 bp DNA ladder.

Table 3 Characteristics of 17 SSR markers based on the results of analysis using 44 tropical soybean advanced lines

SSR markers	Allele number	Allele size (bp)	Major allele frequency	Gene diversity	Heterozigosity	PIC[a]
satt002	11	151-164	0.20	0.86	0.02	0.84
satt009	28	160-244	0.11	0.94	0.43	0.94
satt030	21	169-205	0.13	0.93	0.75	0.93
satt038	17	174-197	0.11	0.92	0.00	0.91
satt045	23	148-179	0.13	0.93	0.91	0.93
satt063	15	118-181	0.32	0.84	0.55	0.83
satt114	19	87-132	0.16	0.91	0.66	0.90
satt147	27	173-236	0.10	0.95	0.70	0.95
satt191	39	202-263	0.07	0.96	0.91	0.96
satt194	11	251-263	0.16	0.88	0.00	0.86
satt197	19	147-235	0.43	0.78	0.36	0.77
satt294	16	266-305	0.14	0.92	0.00	0.91
satt308	26	131-204	0.13	0.94	1.00	0.93
satt431	33	202-280	0.10	0.95	0.98	0.95
satt463	19	155-279	0.33	0.82	0.27	0.80
satt607	14	226-241	0.17	0.90	0.02	0.89
satt646	39	179-237	0.07	0.96	0.95	0.96
Total	377					
Average	22.18		0.17	0.91	0.50	0.90

Remaks: [a]PIC = Polymorphism Information Content.and bp = base pair.

indicated by the abundance of SSR alleles found in this study (Epstein *et al*. 2023). The abundance of alleles provides evidence for this. Previous research on genetic diversity in soybean mutants by Asadi *et al*. (2020) found that the lowest number of SSR alleles (9 alleles) resulted in a genetic diversity of 79%, while the highest number of SSR alleles (28 alleles) resulted in a genetic diversity of 96%.

The average frequency of the major allele generated by this study was 0.17, ranging from 0.07 (*satt191* and *satt646*) to 0.43 (*satt197*). Lower frequencies of the major allele indicate a higher diversity at that locus (Pardeshi *et al*. 2023). The majority of the SSR loci examined in this study had an average major allele frequency of 0.17, indicating significant variability across the genotypes (Table 3). The proportion of genetic diversity, which measures the amount of genetic variety in a population, ranges from 78% (*satt197*) to 96% (*satt191* and *satt646*), with an average of 91%. All SSR markers can detect heterozygous alleles, and their values range from 0.00 (*satt038*, *satt194*, and *satt294*) to 1.00 (*satt308*). The *satt038* marker has a heterozygosity value of 1, indicating that the allele is completely heterozygous (Widaningsih *et al*. 2014).

In linkage studies, the PIC value is the widely preferred index for measuring the discriminating power of a marker or measuring the informativeness of a genetic marker. The PIC value is proportional to the likelihood that individuals would become heterozygous at the locus, while homozygous is not informative (Das *et al*. 2015). PIC values and genetic diversity have a positive correlation (Hossain *et al*. 2020; Mukuze *et al*. 2020). In this study, the PIC values varied from 0.77 (*satt197*) to 0.96 (*satt191* and *satt646*), with an average of 0.90 (Table 3). All SSR markers showed a PIC value of ≥0.50, indicating increased genetic differentiation across soybean genotypes studied. Six markers have a PIC value of <0.90: *satt002*, *satt063*, *satt194*, *satt197*, *satt463*, and *satt607*. Six markers have PIC values ranging from 0.90 to 0.93: *satt030*, *satt038*, *satt045*, *satt114*, *satt294*, and *satt308*. Five markers, *satt009*, *satt646*, *satt147*, *satt431*, and *satt191*, have a PIC of ≥0.94. These markers have the potential to be employed in comprehensive genome mapping research, genetic mapping and QTL analysis, and genomic-assisted selection in soybean breeding programs.

Several studies have highlighted the genetic variety of soybean genotypes (Kumar *et al*. 2022; Denwar *et al*. 2009). Tasma *et al*. (2018) investigated genetic diversity in tropical soybean genotypes (F2) and discovered that the PIC of 27 SSR markers ranged between 0.87 and 0.96, with an average of 0.94. Ullah *et al*. (2021) found PIC ranging from 0.12 to 0.58, with a mean of 0.37. Kumar *et al*. (2022) found that PIC for SSR markers ranged from 0.064 to 0.689, with an average of 0.331. Bisen *et al*. (2015) reported that PIC ranged from 0.049 to 0.526 among genotypes, with an average of 0.199. Khanande *et al*. (2016) found that PIC ranged from 0.33 to 0.83, with an average of 0.55. Furthermore, Wang *et al*. (2006) discovered that PIC ranged between 0.05 and 0.92, with an average of 0.78. These studies revealed a wide range of PIC, demonstrating that soybean genotype diversity varies greatly depending on the number of SSR markers employed and the number of soybean genotypes evaluated in each study.

Phylogenetic Analysis

Phylogenetic analysis of the 44 tropical soybean advanced lines based on 17 SSR markers in this study resulted in a genetic similarity level of 83% (Figure 2). The two main clusters were found based on their genetic similarities.. Cluster I consists of ten genotypes.

This cluster is further subdivided into two subcluste

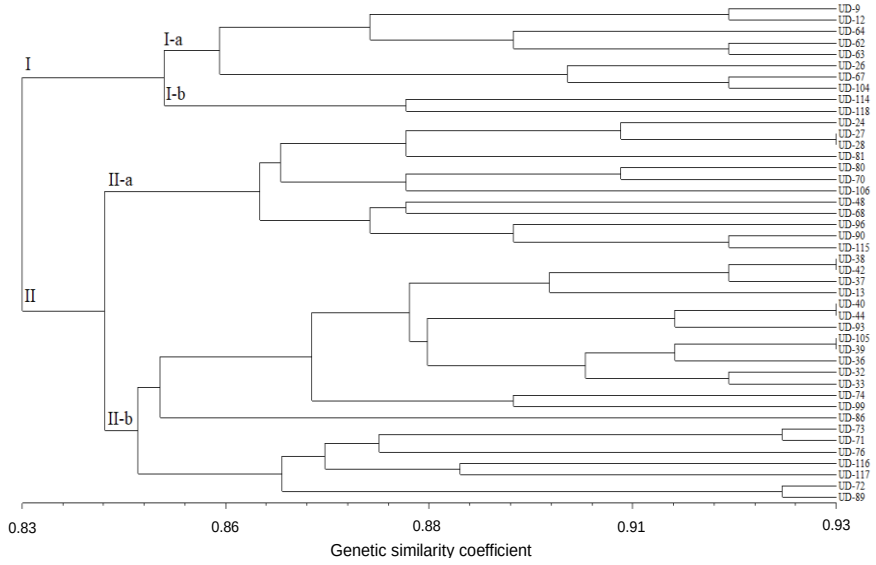

Figure 2 Phylogenetic tree of 44 tropical soybean advanced lines based on analysis results using 17 SSR markers.

Table 4 Genetic similarity matrix of 44 tropical soybean advanced lines based on analysis results using 17 SSR markers

G	UD-9	UD-24	UD-26	UD-27	UD-28	UD-38	UD-40	UD-44	UD-48	UD-64	UD-67	UD-68	UD-73	UD-74	UD-76	UD-80	UD-93	UD-96	UD-99	UD-105	UD-114	UD-116	UD-12	UD-13	UD-32	UD-33	UD-36	UD-37	UD-39	UD-42	UD-62	UD-63	UD-70	UD-71	UD-72	UD-81	UD-86	UD-89	UD-90	UD-104	UD-106	UD-115	UD-117
UD-24	0.84																																										
UD-26	0.89	0.86																																									
UD-27	0.84	0.91	0.83																																								
UD-28	0.87	0.91	0.84	0.93																																							
UD-38	0.86	0.82	0.86	0.84	0.84																																						
UD-40	0.84	0.83	0.84	0.84	0.84	0.93																																					
UD-44	0.86	0.86	0.88	0.86	0.86	0.92	0.93																																				
UD-48	0.84	0.84	0.83	0.84	0.86	0.86	0.86	0.84																																			
UD-64	0.88	0.83	0.89	0.84	0.86	0.86	0.82	0.86	0.87																																		
UD-67	0.86	0.87	0.91	0.82	0.83	0.86	0.84	0.88	0.87	0.88																																	
UD-68	0.86	0.87	0.80	0.88	0.87	0.83	0.83	0.84	0.88	0.86	0.86																																
UD-73	0.83	0.88	0.80	0.82	0.83	0.84	0.83	0.82	0.83	0.80	0.83	0.88																															
UD-74	0.82	0.83	0.82	0.80	0.82	0.88	0.88	0.87	0.83	0.79	0.84	0.86	0.89																														
UD-76	0.80	0.84	0.83	0.86	0.86	0.86	0.88	0.86	0.86	0.84	0.80	0.86	0.87	0.87																													
UD-80	0.82	0.88	0.82	0.87	0.87	0.87	0.86	0.89	0.84	0.84	0.84	0.89	0.88	0.88	0.88																												
UD-93	0.84	0.84	0.86	0.84	0.82	0.89	0.92	0.91	0.86	0.84	0.86	0.86	0.86	0.89	0.89	0.86																											
UD-96	0.82	0.83	0.83	0.86	0.83	0.87	0.84	0.88	0.88	0.88	0.88	0.87	0.82	0.83	0.87	0.87	0.88																										
UD-99	0.80	0.84	0.82	0.80	0.80	0.88	0.88	0.87	0.84	0.82	0.83	0.82	0.87	0.89	0.87	0.83	0.91	0.84																									
UD-105	0.87	0.88	0.88	0.86	0.88	0.86	0.87	0.88	0.84	0.84	0.87	0.83	0.84	0.87	0.84	0.87	0.88	0.86	0.84																								
UD-114	0.83	0.84	0.87	0.84	0.83	0.80	0.80	0.83	0.83	0.84	0.87	0.83	0.80	0.82	0.80	0.82	0.80	0.84	0.80	0.87																							
UD-116	0.80	0.82	0.83	0.80	0.82	0.86	0.83	0.84	0.80	0.84	0.84	0.83	0.84	0.83	0.88	0.88	0.83	0.87	0.86	0.84	0.82																						
UD-12	0.92	0.83	0.87	0.86	0.82	0.84	0.83	0.83	0.84	0.87	0.87	0.84	0.80	0.83	0.79	0.82	0.84	0.83	0.79	0.86	0.89	0.79																					
UD-13	0.87	0.83	0.84	0.84	0.87	0.89	0.84	0.87	0.83	0.83	0.84	0.86	0.84	0.88	0.83	0.87	0.83	0.83	0.82	0.87	0.86	0.84	0.87																				
UD-32	0.82	0.84	0.84	0.84	0.84	0.84	0.84	0.87	0.80	0.82	0.84	0.82	0.86	0.88	0.82	0.86	0.84	0.84	0.83	0.93	0.87	0.84	0.84	0.88																			
UD-33	0.84	0.82	0.83	0.82	0.82	0.87	0.89	0.89	0.79	0.80	0.82	0.80	0.86	0.89	0.86	0.86	0.88	0.82	0.86	0.89	0.83	0.82	0.86	0.87	0.92																		
UD-36	0.88	0.86	0.86	0.83	0.86	0.88	0.89	0.91	0.84	0.83	0.84	0.84	0.84	0.88	0.84	0.86	0.89	0.86	0.87	0.93	0.86	0.82	0.87	0.88	0.89	0.91																	
UD-37	0.91	0.84	0.84	0.83	0.87	0.91	0.87	0.86	0.86	0.84	0.86	0.84	0.88	0.88	0.86	0.84	0.88	0.84	0.84	0.91	0.82	0.83	0.88	0.91	0.88	0.89	0.89															
UD-39	0.87	0.84	0.87	0.83	0.86	0.88	0.92	0.88	0.86	0.84	0.88	0.83	0.86	0.89	0.88	0.86	0.91	0.84	0.86	0.93	0.84	0.84	0.87	0.86	0.88	0.91	0.89	0.93														
UD-42	0.87	0.82	0.86	0.83	0.84	0.93	0.88	0.88	0.84	0.86	0.86	0.84	0.87	0.87	0.84	0.84	0.89	0.84	0.86	0.88	0.82	0.84	0.87	0.89	0.88	0.89	0.91	0.93	0.91													
UD-62	0.84	0.82	0.87	0.84	0.82	0.80	0.79	0.82	0.83	0.89	0.87	0.83	0.78	0.80	0.80	0.79	0.83	0.88	0.80	0.83	0.91	0.80	0.89	0.79	0.83	0.80	0.80	0.82	0.83	0.83												
UD-63	0.87	0.87	0.84	0.89	0.86	0.82	0.80	0.83	0.82	0.89	0.84	0.84	0.80	0.80	0.82	0.83	0.84	0.87	0.82	0.84	0.84	0.80	0.91	0.80	0.83	0.83	0.83	0.86	0.84	0.83	0.92											
UD-70	0.83	0.87	0.86	0.88	0.87	0.83	0.84	0.88	0.80	0.84	0.86	0.86	0.83	0.86	0.87	0.91	0.87	0.88	0.82	0.91	0.86	0.86	0.83	0.89	0.89	0.87	0.87	0.86	0.88	0.83	0.83	0.86										
UD-71	0.80	0.86	0.81	0.84	0.81	0.88	0.84	0.84	0.82	0.85	0.82	0.85	0.93	0.85	0.89	0.88	0.88	0.88	0.88	0.84	0.81	0.88	0.80	0.85	0.85	0.84	0.82	0.86	0.84	0.86	0.80	0.82	0.86									
UD-72	0.80	0.83	0.86	0.82	0.83	0.84	0.84	0.84	0.80	0.87	0.86	0.79	0.86	0.87	0.88	0.84	0.87	0.86	0.88	0.87	0.83	0.86	0.82	0.82	0.84	0.87	0.83	0.86	0.91	0.84	0.84	0.87	0.87	0.89								
UD-81	0.83	0.87	0.82	0.89	0.88	0.84	0.84	0.87	0.84	0.83	0.86	0.87	0.84	0.84	0.84	0.89	0.84	0.84	0.80	0.87	0.86	0.84	0.87	0.86	0.87	0.84	0.86	0.84	0.87	0.86	0.83	0.86	0.86	0.82	0.84							
UD-86	0.86	0.82	0.86	0.79	0.80	0.82	0.84	0.84	0.83	0.80	0.83	0.82	0.83	0.86	0.80	0.82	0.84	0.82	0.82	0.88	0.83	0.80	0.84	0.86	0.89	0.88	0.86	0.86	0.86	0.83	0.80	0.79	0.86	0.81	0.82	0.79						
UD-89	0.81	0.85	0.86	0.86	0.84	0.86	0.86	0.86	0.84	0.86	0.85	0.82	0.82	0.84	0.88	0.85	0.91	0.89	0.86	0.88	0.82	0.84	0.82	0.82	0.84	0.85	0.86	0.86	0.89	0.86	0.82	0.86	0.88	0.88	0.93	0.85	0.81					
UD-90	0.87	0.89	0.83	0.89	0.88	0.84	0.84	0.86	0.86	0.82	0.86	0.89	0.86	0.84	0.84	0.87	0.86	0.88	0.80	0.91	0.87	0.83	0.87	0.88	0.88	0.86	0.88	0.91	0.88	0.86	0.84	0.87	0.91	0.85	0.83	0.89	0.86	0.86				
UD-104	0.84	0.83	0.89	0.79	0.82	0.89	0.86	0.89	0.80	0.86	0.92	0.83	0.84	0.88	0.80	0.83	0.88	0.84	0.87	0.86	0.83	0.84	0.83	0.87	0.86	0.86	0.86	0.87	0.87	0.89	0.84	0.83	0.86	0.84	0.86	0.83	0.83	0.84	0.82			
UD-106	0.84	0.86	0.82	0.84	0.84	0.86	0.86	0.88	0.86	0.84	0.88	0.85	0.88	0.86	0.85	0.90	0.86	0.86	0.82	0.85	0.80	0.84	0.84	0.85	0.86	0.89	0.85	0.88	0.86	0.85	0.78	0.84	0.86	0.86	0.85	0.88	0.86	0.86	0.86	0.86		
UD-115	0.83	0.87	0.82	0.87	0.87	0.82	0.82	0.83	0.88	0.86	0.86	0.88	0.86	0.83	0.86	0.87	0.83	0.91	0.80	0.84	0.86	0.82	0.83	0.83	0.84	0.83	0.84	0.86	0.83	0.82	0.87	0.87	0.86	0.84	0.82	0.87	0.83	0.84	0.92	0.82	0.89	
UD-117	0.80	0.82	0.84	0.82	0.81	0.85	0.84	0.84	0.80	0.82	0.86	0.84	0.88	0.86	0.86	0.85	0.86	0.86	0.86	0.84	0.85	0.89	0.82	0.86	0.86	0.84	0.84	0.85	0.85	0.84	0.81	0.82	0.88	0.89	0.89	0.88	0.82	0.88	0.86	0.88	0.87	0.85	
UD-118	0.82	0.82	0.83	0.86	0.83	0.82	0.80	0.78	0.83	0.84	0.83	0.84	0.82	0.79	0.83	0.82	0.82	0.83	0.80	0.80	0.88	0.83	0.86	0.80	0.80	0.80	0.78	0.80	0.80	0.83	0.89	0.86	0.80	0.82	0.82	0.86	0.80	0.81	0.83	0.83	0.84	0.87	0.85

This cluster is further subdivided into two subclusters: IA and IB. The IA subcluster has eight genotypes: UD-9, UD-12, UD-64, UD-62, UD-63, UD-26, UD-67, and UD-104. The IB subcluster has only two genotypes: UD-114 and UD-118. The five genotypes in sub-cluster IA: UD-9, UD-12, UD-64, UD-62, and UD-63, are all produced from the same cross, Grobogan × Melrose (Table 1), hence the lines have the same genetic background. Cluster II contains 34 genotypes. This cluster is further divided into two sub-clusters: IIA and IIB. Sub-cluster IIA comprises 12 genotypes, while sub-cluster IIB has 22 genotypes (Figure 2). Several genotypes are classified based on the same genetic background (Table 1).

The genetic similarity between genotype pairs ranged from 0.78 to 0.93 (Table 4), indicating the genetically diverse nature of the lines. This variety of similarities is conceivable since the genotypes investigated in this study were derived from five separate pairings of crossings between five distinct male parents (Melrose, Vernal, Glycine H, Paranagoiana, and Hinson lj) and one female parent of the same female (Grobogan) (Tasma *et al*. 2018). Genetic similarity between 0.78 and 0.80 occurred in 8.56% (81 genotype pairings) of the genotypes tested. This shows that the genotypes are genetically distinct, which could be attributed to a combination of diverse parent genomes. The genotype with the highest genetic similarity (0.93) in 11 genotype pairs had a very close relationship and originated from the same cross. This genetic similarity result suggests that genotype pairs are 93% identical, with a 7% difference.

The results of grouping in phylogenetic analysis studies can be utilized to pick parents in breeding operations. In this study, phylogenetic analysis identified two major clusters with an 83% genetic similarity (Figure 2). Previous research has found 75% genetic similarity with two main clusters (Lestari *et al*. 2021), 76% with two main clusters (Tasma *et al*. 2018), and 57% with three significant clusters (Pardeshi *et al*. 2023). The discrepancies in genetic similarity across the research listed above were caused by variances in the types and numbers of soybean genotypes evaluated, as well as the types and numbers of SSR markers employed in each study. According to Hossain *et al*. (2020), the value of genetic similarity can be utilized to estimate the degree of link between the genotypes under consideration.

The important of diversity in plant breeding initiatives should not be underestimated (Wibisono *et al*. 2022). The measurement of diversity in a certain plant provides basic data for selecting parental lines in a plant breeding program. Crossover between genotypes from the same cluster is undesirable since it does not result in the desired segregants. When genotypes with identical genetic traits are clustered together, it implies a lack of diversity (Kachare *et al*. 2020). In contrast, genotypes with larger genetic spacing, such as those represented by various clusters, indicate higher diversity among the clusters.

Creation of DNA Barcoding

This study analyzed the DNA barcoding profile using five efficient and informative SSR markers: *satt009, satt646, satt147, satt431*, and *satt191*, with a PIC of ≥0.94 (Tables 5 and 6). These SSR markers were able to differentiate all 44 soybean genotypes studied (Figure 3), as evidenced by phylogenetic trees comparable to those created using all 17 SSR markers (Figure 2). Five selected SSR markers were utilized to generate IDs in the form of numerical codes. This numeric code is referred to as the genotype's "barcode". Barcodes are generated based on the allele size range (Table 5). The allele size range of each SSR

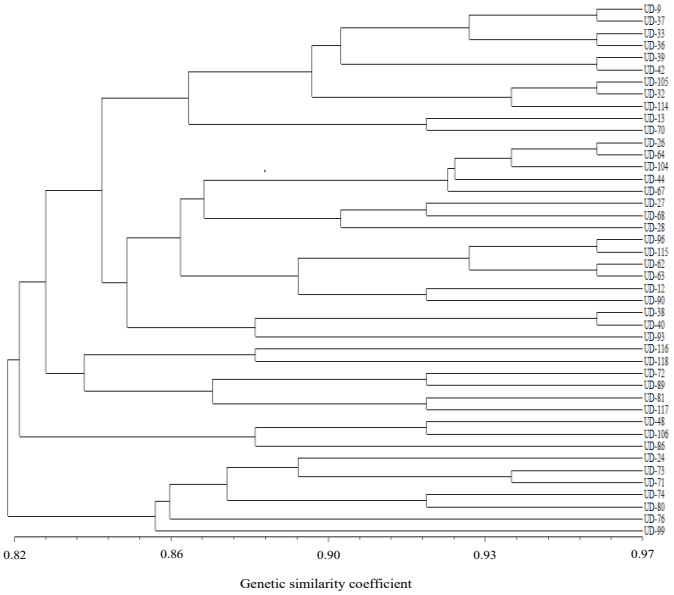

Figure 3 SSR Clustering of 44 tropical soybean advanced lines based on selected 5 SSR markers *(satt009, satt646, satt147, satt431, and satt191).*

marker was calculated using GelAnalyzer software (Table 6). Numerical codes were used to genetically differentiate each genotype utilizing a digitizing technique. For example, the SSR markers *satt009* produced 14 integer codes that represent the amplified homozygous allele sizes (Table 5). Numerical codes were used to generate genotype IDs (Table 7). The employment of a digital code system to create genetic identification protects genotypes from counterfeiting, theft of genetic material, and the protection of protected superior genotypes (Lestari *et al.* 2021).

DNA barcodes have been successfully developed utilizing SSR marker data from a variety of plant species. For example, Chinnappareddy *et al*. (2012) successfully established a DNA barcode for eggplant, whereas Kanupriya *et al*. (2011) created a DNA barcode for guava. Previous researchers have also concentrated on the creation of barcodes with SSR marks on soybeans. In one study, 102 soybean varieties from India were examined using 10 highly polymorphic SSR markers chosen specifically for their PIC (Rani *et al*. 2016). These ten SSR markers are utilized to generate a barcode with a specific 10-digit number that acts as the identifying code for each soybean variety. Harisha *et al*. (2021) also shown how to translate allele variations seen on 53 SSR markers into DNA barcode profiles by separating allele sizes at each SSR locus. Sohn *et al*. (2017) proposed a

Table 5 The code of each allele size range of the five selected SSR markers (*satt009*, *satt646*, *satt147*, *satt431*, and *satt191*) used to develop DNA barcoding profiles of the 44 tropical soybean advanced lines

Code	Allele size ranges				
	satt009	*satt646*	*satt147*	*satt431*	*satt191*
00	160-163	179-182	173-176	202-205	202-205
01	164-167	183-186	177-180	206-209	206-209
02	168-171	187-190	181-184	210-213	210-213
03	172-175	191-194	185-188	214-217	214-217
04	176-179	195-198	189-192	218-221	218-221
05	180-183	199-202	193-196	222-225	222-225
06	184-187	203-206	197-200	226-229	226-229
07	188-191		201-204	230-233	
08	192-195		205-208	234-237	
09	196-199		209-212	238-241	
10	200-203		213-216		
11	204-207				
12	208-211				
13	212-215				

Table 6 Allele sizes of each selected SSR marker (*satt009*, *satt646*, *satt147*, *satt431*, and *satt191*) based on analysis using *GelAnalyzer* software

Genotype	Allele size (bp)					Genotype	Allele size (bp)				
	satt 009	*satt 646*	*satt 147*	*satt 431*	*satt 191*		*satt 009*	*satt 646*	*satt 147*	*satt 431*	*satt 191*
UD-9	161	195	208	205	209	UD-12	163	199	207	202	210
UD-24	213	196	209	238	214	UD-13	167	201	206	237	209
UD-26	161	195	180	206	207	UD-32	215	200	205	206	208
UD-27	167	196	213	210	211	UD-33	215	184	207	205	208
UD-28	168	196	214	209	206	UD-36	212	200	207	205	207
UD-38	164	180	214	211	206	UD-37	162	186	205	205	207
UD-40	162	179	214	210	202	UD-39	162	185	205	206	205
UD-44	163	195	211	212	207	UD-42	161	183	206	206	207
UD-48	212	191	209	211	205	UD-62	162	195	173	207	210
UD-64	163	196	176	209	208	UD-63	162	198	175	204	210
UD-67	163	194	211	209	215	UD-70	167	198	206	235	220
UD-68	168	198	209	241	213	UD-71	213	184	174	210	222
UD-73	215	184	209	241	224	UD-72	166	184	174	206	222
UD-74	169	199	211	241	225	UD-81	165	199	209	206	210
UD-76	166	186	178	212	225	UD-86	210	193	208	221	209
UD-80	167	206	210	241	219	UD-89	166	184	175	229	210
UD-93	163	186	211	212	212	UD-90	165	195	207	203	213
UD-96	211	196	176	210	212	UD-104	160	198	212	208	208
UD-99	215	187	209	211	228	UD-106	209	191	210	204	214
UD-105	215	204	208	209	209	UD-115	209	197	176	204	210
UD-114	215	201	208	206	212	UD-117	164	202	180	233	212
UD-116	169	204	179	208	227	UD-118	210	203	215	206	212

barcode technique for soybean genetic identification that uses particular InDel markers.

These barcode profiles (Table 7) aid in the discovery of genotypic variances, making them an invaluable tool for exact identification of individuals. These barcode profiles also serve as a reference or standard for DNA barcode libraries (Kanupriya *et al*. 2011), defend intellectual property rights such as plant variety protection (Diwan and Cregan 1997) and settle economic disputes (Jian *et al.* 2014). The barcode construction employing a mix of SSR markers gives methodological advice for creating a uniform DNA barcode database and carrying out future mapping assessments of soybean varieties. Molecular characterisation using polyacrylamide gel electrophoresis is predicted to increase the accuracy and precision of soybean variety identification. Many studies have demonstrated the utility of barcodes created in soybean breeding programs for selecting pure lines during the breeding process (Rani *et al*. 2016; Harisha *et al*. 2021). We expect that the genetic barcodes obtained from our research will be useful in genetic analysis and soybean breeding efforts targeted at improving variety. In the future, it will be extremely beneficial to be able to create a barcode system that assigns distinct identities to additional soybean genotypes.

CONCLUSION

SSR marker analysis showed distinctive polymorphism among the 44 tropical soybean advanced lines analysed in this study. A total of 377 alleles were identified, with an average of 22.18 alleles per SSR locus. The polymorphism information content (PIC) ranged from 0.77 to 0.96, with an average of 0.90. The forty-four soybean genotypes examined in this study were grouped into two major clusters based on phylogenetic analysis, with an 83% genetic similarity level. Five markers (*satt009*, *satt646*, *satt147*, *satt431*, and *satt191*) with a PIC of ≥0.94 were found to be informative and acceptable for constructing DNA barcodes. The developed barcode is intended to be useful for DNA fingerprint and protecting the superior lines of tropical soybeans studied in this study.

ACKNOWLEDGEMENT

This research was supported by RIIM-LPDP and BRIN batch III in 2023, as well as the ICABIOGRAD IAARD national budget from the Ministry of Agriculture in 2017–2018. We thank USDA/ARS USA for providing the genetic material utilized in this study.

REFERENCES

Amiteye S. 2021. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliyon*. 7(10): e08093. https://doi.org/10.1016/j.heliyon.2021.e08093

Asadi A, Dewi N, Nugroho K, Terryana RT, Mastur, Lestari P. 2020. Evaluation of SSR and important agronomical characters of promising mutant lines of soybean. *Biodiversitas.* 21(1): 299–310. https://doi.org/10.13057/biodiv/d210137

Barcodes. 2021. Barcode marker online. Barcodes Inc, Chicago (US). https://www.barcodesinc.com/generator/index.ph

Bisen A, Khare D, Nair P, Tripathi N. 2015. SSR analysis of 38 genotypes of soybean (*Glycine max* (L.) Merr.) genetic diversity in India. *Physiology and Molecular Biology of Plants*. 21: 109–115. https://doi.org/10.1007/s12298-014-0269-8

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*. 32(3): 314–331.

[BPS] Badan Pusat Statistik. 2021. Impor kedelai menurut negara asal utama. https://www.bps.go.id/

Carpentieri-Pípolo V, Almeida LAD, Kiihl RADS. 2002. Inheritance of a long juvenile period under short-day conditions in soybean. *Genetics and Molecular Biology*. 25: 463–469. https://doi.org/10.1590/S1415-47572002000400016

Chinnappareddy LRD, Khandagale K, Reddy SHS, Kanupriya C, Chennareddy A, Singh TH. 2012. SSR-based DNA barcodes as a tool for identification of eggplant genotypes. *International Journal of Vegetable Science*. 18(3): 260–271. https://doi.org/10.1080/19315260.2011.633976

Chung JW, Lee GA, Lee SS, Bang KH, Park CB, Park YJ. 2009. Cultivar discrimination of Korean and Chinese Boxthorn (*Lycium chinense* Mill. and *Lycium barbarum* L.) using SSR markers. *Korean Journal of Medicinal Crop Science*. 17(6): 445–451.

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, VanToai TT, Lohnes DG, Chung J, Specht JE. 1999. An integrated genetic linkage map of the soybean genome. *Crop Science*. 39(5): 1464–1490. https://doi.org/10.2135/cropsci1999.3951464x

Das A, Kesari V, Satyanarayana VM, Parida A, Mitra S, Rangan L. 2015. Genetic diversity in ecotypes of the

Table 7 DNA barcodes of 44 tropical soybean advanced lines developed based on 5 selected SSR markers (*satt009*, *satt646*, *satt147*, *satt431*, and *satt191*)

Genotypes	Code	Barcode	Genotypes	Code	Barcode
UD-9	0004080001		UD-12	0005080002	
UD-24	1304090903		UD-13	0105080801	
UD-26	0004010101		UD-32	1305080101	
UD-27	0104100202		UD-33	1301080001	
UD-28	0204100101		UD-36	1305080001	
UD-38	0100100201		UD-37	0001080001	
UD-40	0000100200		UD-39	0001080100	
UD-44	0004090201		UD-42	0001080101	
UD-48	1303090200		UD-62	0004000102	
UD-64	0004000101		UD-63	0004000002	
UD-67	0003090103		UD-70	0104080804	
UD-68	0204090902		UD-71	1301000205	
UD-73	1301090905		UD-72	0101000105	
UD-74	0205090905		UD-81	0105090102	
UD-76	0101010905		UD-86	1203080401	
UD-80	0106090905		UD-89	0101000602	
UD-93	0001090202		UD-90	0104080002	
UD-96	1204000202		UD-104	0004090101	
UD-99	1302090206		UD-106	1203090003	
UD-105	1306080101		UD-115	1204000002	
UD-114	1305080102		UD-117	0105010702	
UD-116	0206010106		UD-118	1206100802	

scarce wild medicinal crop *Zingiber moran* revealed by ISSR and AFLP marker analysis and chromosome number assessment. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*. 149(1): 111–120. https://doi.org/10.1080/11263504.2013.795197

Denwar NN, Awuku FJ, Diers B, Addae-Frimpomaah F, Chigeza G, Oteng-Frimpong R, Puozaa DK, Barnor MT. 2009. Genetic diversity, population structure and key phenotypic traits driving variation within soyabean (*Glycine max*) collection in Ghana. *Plant Breeding*. 138(5): 577–587. https://doi.org/10.1111/pbr.12700

Diwan N, Cregan PB. 1997. Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. *Theoretical and Applied Genetics*. 95: 723–733. https://doi.org/10.1007/s001220050618

Dong YS, Zhao LM, Liu B, Wang ZW, Jin ZQ, Sun H. 2004. The genetic diversity of cultivated soybean grown in China. *Theoretical and Applied Genetics*. 108: 931–936. https://doi.org/10.1007/s00122-003-1503-x

dos Santos Silva FC, Sediyama T, de Cássia Teixeira Oliveira R, Borém A, da Silva FL, Bezerra ARG, da Silva AF. 2017. Economic importance and evolution of breeding. In: Lopes da Silva F, Borém A, Sediyama T, Ludke W. (eds) *Soybean Breeding*. Swiss (CH): Springer Cham. 1–16. https://doi.org/10.1007/978-3-319-57433-2_1

Doyle JJ, Doyle JL. 1990. A rapid total DNA preparation procedure for fresh plant tissue. *Focus*. 12: 13–15.

Epstein R, Sajai N, Zelkowski M, Zhou A, Robbins KR, Pawlowski WP. 2023. Exploring impact of recombination landscapes on breeding outcomes. *Proceedings of the National Academy of Sciences.* 120(14): e2205785119. https://doi.org/10.1073/pnas.220578511

Gupta S, Agrawal N, Tripathi R, Kumawat G, Nataraj V, Maranna S, Satpute GK, Ratnaparkhe MB, Rajesh V, Chandra S, Jain M. 2021. Long juvenility trait: A

vehicle for commercial utilization of soybean (*Glycine max*) in lower latitudes. *Plant Breeding*. 140(4): 543–560. https://doi.org/10.1111/pbr.12926

Harisha R, Bhadru D, Vanisri S, Gourishanakar V, Satish L. 2021. SSR and morphological traits-based fingerprints and DNA barcodes for varietal identification in rice. *Biotechnology & Biotechnological Equipment*. 35(1): 1461–1473. https://doi.org/10.1080/13102818.2021.1987324

Hartwig EE, Kiihl RA. 1979. Identification and utilization of a delayed flowering character in soybeans for short-day conditions. *Field Crops Research*. 2: 145–151. https://doi.org/10.1016/0378-4290(79)90017-0

Hossain MA, Islam MM, Emon RM, Rana MS, Hossain MA, Uddin MI, Malek M A, Khan NA, Nuruzzaman M. 2020. Microsatellite-based DNA fingerprinting and genetic analysis of some selected aus rice (*Oryza sativa* L.) genotypes. *Annals of Agricultural & Crop Sciences*. 5(3): 1066–1075.

Jian C, Deyi Q, Qiaoyun Y, Jia H, Dexing L, Xiaoya W, Leiqing Z. 2014. A successful case of DNA barcoding used in an international trade dispute. *DNA Barcodes*. 2(1): 21–28. https://doi.org/10.2478/dna-2014-0004

Kachare S, Tiwari S, Tripathi N, Thakur VV. 2020. Assessment of genetic diversity of soybean (*Glycine max*) genotypes using qualitative traits and microsatellite markers. *Agricultural Research*. 9(1): 23–34. https://doi.org/10.1007/s40003-019-00412-y

Kanupriya, Latha PM, Aswath C, Reddy L, Padmakar B, Vasugi C, Dinesh MR. 2011. Cultivar identification and genetic fingerprinting of guava (*Psidium guajava*) using microsatellite markers. *International Journal of Fruit Science*. 11: 184–196. http://dx.doi.org/10.1080/15538362.2011.578521

Khanande AS, Jadhav PV, Kale PB, Madavi SM, Mohari MP, Nichal SS. 2016. Genetic diversity in vegetable and grain type soybean genotypes identified using morphological descriptor and EST-SSR markers. *Vegetos-An International Journal of Plant Research*. 29(3): 158–173. https://doi.org/10.5958/2229-4473.2016.00085.9

Kumar SJ, Susmita C, Sripathy KV, Agarwal DK, Pal G, Singh AN, Kumar S, Rai AK, Simal-Gandara J. 2022. Molecular characterization and genetic diversity studies of Indian soybean (*Glycine max* (L.) Merr.) cultivars using SSR markers. *Molecular Biology Reports*. 49: 2129–2140. https://doi.org/10.1007/s11033-021-07030-4

Lazer IJ, Horvath-Lazar E. 2010. Gel Analyzer 2010a: freeware 1D gel electrophoresis image analysis software. http://www.gelanalyzer.com/index.html

Lestari P, Putri RE, Rineksane IA, Handayani E, Nugroho K, Terryana RT. 2021. Keragaman genetik 27 aksesi Kedelai (*Glycine max* l. Merr.) introduksi subtropis berdasarkan marka SSR. *Vegetalika*. 10(1): 1–17. https://doi.org/10.22146/veg.58418

Li X, Yang Y, Henry R J, Rossetto M, Wang Y, Chen S. 2015. Plant DNA barcoding: from gene to genome. *Biological Reviews*. 90(1): 157–166. https://doi.org/10.1111/brv.12104

Liu J, Li M, Zhang Q, Wei X, Huang X. 2020. Exploring the molecular basis of heterosis for plant breeding. *Journal of Integrative Plant Biology*. 62(3): 287–298. https://doi.org/10.1111/jipb.12804

Liu K, Muse SV. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*. 21(9): 2128–2129. https://doi.org/10.1093/bioinformatics/bti282

Liu Z, Li H, Wang X, Zhang Y, Gou Z, Zhao X, Ren H, Wen Z, Li Y, Yu L, Gao H, Wang D, Qi X, Qiu L. 2023. QTL for yield per plant under water deficit and well-watered conditions and drought susceptibility index in soybean (*Glycine max* (L.) Merr.). *Biotechnology & Biotechnological Equipment*. 37(1): 92–103. https://doi.org/10.1080/13102818.2022.2155569

Lu S, Zhao X, Hu Y, Liu S, Nan H, Li X, Fang C, Cao D, Shi X, Kong L, Su T, Zhang F, Li S, Wang Z, Yuan X, Cober ER, Weller JL, Liu B, Hou X, Tian Z, Kong F. 2017. Natural variation at the soybean J locus improves adaptation to the tropics and enhances yield. *Nature Genetics*. 49(5): 773–779. https://doi.org/10.1038/ng.3819

Manjarrez-Sandoval P, Carter Jr TE, Webb DM, Burton JW. 1997. Heterosis in soybean and its prediction by genetic similarity measures. *Crop Science*: 37(5): 1443–1452. https://doi.org/10.2135/cropsci1997.0011183X003700050005x

Mukuze C, Tukamuhabwa P, Maphosa M, Dari S, Dramadri IO, Obua T, Kongai H, Rubaihayo P. 2020. Genetic diversity analysis among soybean genotypes using SSR markers in Uganda. *African Journal of Biotechnology*. 19(7): 439–448. https://doi.org/10.5897/AJB2020.17152

Pardeshi P, Jadhav P, Sakhare S, Zunjare R, Rathod D, Sonkamble P, Saroj R, Varghese P. 2023. Morphological and microsatellite marker-based characterization and diversity analysis of novel vegetable soybean [*Glycine max* (L.) Merrill]. *Molecular Biology Reports*. 50(5): 4049–4060. https://doi.org/10.1007/s11033-023-08328-1

Presello DA, Reid LM, Butler G, Mather DE. 2005. Pedigree selection for Gibberella ear rot resistance

in maize. *Euphytica*. 143: 1–8. https://doi.org/10.1007/s10681-005-6149-0

Rani A, Kumar V. Shukla S, Jha P, Rawal R. 2016. DNA barcoding of Indian soybean varieties as constructed through SSR markers. *Seed Science and Technology*. 44(2): 357–369. https://doi.org/10.15258/sst.2016.44.2.18

Ray JD, Hinson K, Mankono JEB, Malo MF. 1995. Genetic control of a long-juvenile trait in soybean. *Crop Science*. 35(4): 1001–1006. https://doi.org/10.2135/cropsci1995.0011183X003500040012x

Rohlf FJ. 2000. *NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, version 2. 1*. Setauket (US): Exeter Publishing.

Sambrook J, Fritch EF, Maniatis T. 1989. *Molecular cloning: a laboratory manual.* Long Island (US): Cold Spring Harbor Laboratory Press.

Singh AK, Chaurasia S, Kumar S, Singh R, Kumari J, Yadav MC, Singh N, Gaba S, Jacob SR. 2018. Identification, analysis and development of salt responsive candidate gene based SSR markers in wheat. *BMC Plant Biology*. 18(1): 1–15. https://doi.org/10.1186/s12870-018-1476-1

Sohn HB, Kim SJ, Hwang TY, Park HM, Lee YY, Markkandan K, Lee D, Lee S, Hong S Y, Song YH, Koo BC, Kim YH. 2017. Barcode system for genetic identification of soybean [*Glycine max* (L.) Merrill] cultivars using InDel markers specific to dense variation blocks. *Frontiers in Plant Science*. 8: 1–12. https://doi.org/10.3389/fpls.2017.00520

Spehar CR. 1995. Impact of strategic genes in soybean on agricultural development in the Brazilian tropical savannahs. *Field Crops Research*. 41(3): 141–146. https://doi.org/10.1016/0378-4290(95)00007-D

Tasma IM, Lorenzen LL, Green DE, Shoemaker RC. 2001. Mapping genetic loci for flowering time, maturity, and photoperiod insensitivity in soybean. *Molecular Breeding*. 8: 25–35. https://doi.org/10.1023/A:1011998116037

Tasma IM, Shoemaker RC. 2003. Mapping flowering time gene homologs in soybean and their association with maturity (E) loci. *Crop Science*. 43(1): 319–328. https://doi.org/10.2135/cropsci2003.3190

Tasma IM, Yani NMG, Purwaningdyah R, Satyawan D, Nugroho K, Lestari P, Kurniawan R, Trijatmiko, Mastur. 2018. Genetic diversity analysis and F_2 population development for breeding of long juvenile trait in soybean. *Jurnal AgroBiogen*. 14(1): 11–22.

Ullah A, Akram Z, Malik SI, Khan KSU. 2021. Assessment of phenotypic and molecular diversity in soybean [*Glycine max* (L.) Merr.] germplasm using morpho-biochemical attributes and SSR markers. *Genetic Resources and Crop Evolution*. 68: 2827–2847. https://doi.org/10.1007/s10722-021-01157-w

Valentini A, Pompanon F, Taberlet P. 2009. DNA barcoding for ecologists*. Trends in Ecology & Evolution.* 24(2): 110–117. https://doi.org/10.1016/j.tree.2008.09.011

Wang L, Guan R, Zhang XL, Chang R, Qiu L. 2006. Genetic diversity of Chinese cultivated soybean revealed by SSR markers. *Crop Science*. 46(3): 1032–1038. https://doi.org/10.2135/cropsci2005.0051

Wibisono K, Aisyah SI, Nurcholis W, Suhesti S. 2022. Sensitivity in callus tissue of *Plectranthus amboinicus* (L.) through mutation induction with colchicine. *Agrivita Journal of Agricultural Science*. 44(1): 82–95. http://doi.org/10.17503/agrivita.v44i1.3058

Widaningsih NA, Purwanto E, Nandariyah N, Reflinur R. 2014. The use of DNA microsatellite markers for genetic diversity identification of soybean (*Glycine max* (L) Meriil.) as a supplementary method in reference collections management. *Indonesian Journal of Biotechnology*. 19(2): 136–145. https://doi.org/10.22146/ijbiotech.9306