Research article

Diversity Analysis of 53 Soybean Accessions Introduced from China Based on Morphological Characteristics and SSR Markers

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Abstract

Indonesia still faces challenges in meeting its national soybean demand. Genetic diversity can provide new resources to improve soybean production and quality. Genetic diversity of 53 soybean accessions introduced from China, based on morphological characteristics and 17 SSR markers, was analyzed in this study. Principal component analysis (PCA) conducted on morphological characters produced a total diversity value of 64.67% and identified four main components. Based on phylogenetic analysis and principal coordinate analysis (PCoA) two accessions showed low genetic similarity of 78% (China cult-55 and Mi yang niu mao huang), which indicated that they could be selected as parents for plant breeding programs. In addition, 772 SSR alleles at an average of 45 alleles per locus were detected. The average heterozygosity was 0.83, and the average polymorphic information content (PIC) value was 0.96. All SSR markers showed a PIC value > 0.8, indicating their informativeness in analyzing genetic diversity of soybean. The phylogenetic analysis indicated a genetic similarity of 82% and the accessions were grouped into two main clusters. The phylogenetic analysis depicted that several accessions could be grouped based on the growth type and origin. The results of morphological characterization and molecular markers in the analysis of genetic diversity are beneficial for selecting parental crosses when developing new varieties.

Keywords

genetic diversity;
morphology;
principal component analysis;
principal coordinate analysis;
soybean;
SSR marker

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1. Introduction

Soybean is one of the three main food commodities in Indonesia, after rice and corn. Soybean protein content ranged from 35.1-42% [1]. Soybeans with high protein content can be used to meet the nutrition requirements of the population [2]. In Indonesia, soybeans are often consumed in processed forms such as tofu, tempeh, soy sauce, soy milk, etc. [3, 4]. Soybean is a plant with a taproot system, two types of stem growth (determinate and indeterminate), and a trifoliate leaf. Soybean is a self-pollinating plant and has two flower colors (white and purple). The number of seeds per pod produced in soybean ranges from 1-5 seeds and the color of the pods when young is light green and changes to dark brown when pods are mature [5-8]. In 2018, Indonesia's soybean production was 982,598 tons with a productivity of 1.44 tons/ha [9]. However, this production was unable to meet the national soybean demand, which averaged at 2.3 million tons/year. Therefore, Indonesia's dependency on soybean imports increased from 69.7% of domestic consumption in 2013 to 88.1% in 2019 [10]. To achieve soybean self-sufficiency, soybean productivity needs to be increased through plant breeding to develop new superior varieties [11]. The development of new superior varieties can be achieved through various methods including artificial crossing [12, 13], mutation induction [14-17], local variety purification [18], and utilization of introduced varieties [19].

Introduced genotypes with high adaptability such as agronomic performance and higher productivity than local varieties, have the potential to become new superior varieties and can be used as parents in soybean breeding programs [20]. There are several methods that can be used in plant breeding programs, such as hybridization, mutation, genetic transformation, and molecular breeding through the use of marker-assisted selection techniques [21-23]. In previous studies, several plant breeding programs for soybeans were reported. These included soybean breeding for resistance to whitefly [24], molecular breeding to overcome biotic stresses [25], molecular breeding of long juvenile (LJ) trait to improve soybean yield in low latitude tropical regions[12], and mutation breeding methods to develop drought tolerant soybean [22]. Plant introduction (PI) is one of the important sources of targeted traits in soybean breeding programs. For example, LJ trait was obtained by introducing soybean genotypes having LJ character from the USA [12]. The PIs obtained from other countries were intensively tested for their phenotypic performances in the environmental conditions where the soybean variety would be developed. Therefore, for breeding purposes, it is highly necessary to characterize the introduced soybean genotypes to determine their properties both at morphological and molecular levels [26].

Plant morphological characteristics can be used as a reference for identification, mapping of relationships, and taxonomy of plants [27]. However, morphological characters are highly influenced by environmental factors [28], and thus supporting analysis of genetic diversity using molecular markers is needed. Analysis of genetic diversity using molecular markers has advantages because it can be done at early stages of plant growth [29]. In addition, molecular markers are stable, can distinguish between closely related individuals, and are not influenced by environmental factors [30]. Morphological characterization still needs to be done despite its limitations, because an individual selected using molecular markers may not necessarily have the desired morphological character [31, 32]. Therefore, molecular characterization can be used as supporting data for morphological characterization results to obtain comprehensive and complete information on the genetic information of the accession or variety being analyzed.

Molecular markers are parts of DNA sequences scattered throughout the genome that are used to identify genetic differences between organisms or species [33]. In addition to being used for genetic diversity analysis [12], molecular markers can also be used in gene mapping analysis [34, 35], fingerprinting analysis [36], and mutant gene detection [37]. The use of molecular markers in soybean genetic diversity analysis was carried out in previous studies including Rani *et*

al. [38] on 96 soybean accessions with 96 SSR and EST-SSR markers, Jain *et al*. [39] on 24 soybean genotypes with 18 RAPD markers, Sulistyo *et al*. [40] on 40 soybean accessions with 13 SSR markers, Slamet *et al*. [41] on 40 soybean genotypes using 20 SSR markers, and Agam *et al*. [42] on 11 mutant soybean genotypes with 12 RAPD markers.

Simple Sequence Repeat (SSR) is a type of molecular marker with modern techniques that are widely used by researchers. SSR markers are efficient in differentiating soybean accessions that are closely related [43]. SSR markers are widely used in genetic diversity analysis of plants including soybean [12]. SSR markers are codominant, highly reproducible, distributed well throughout the plant genome, highly polymorphic, and easily amplified through regular PCR techniques [44, 45]. All those advantages make SSR markers very popular with researchers.

The use of SSR markers for genetic diversity analyses has been widely carried out in various plant species including oil palm [46], rice [47, 48], mung bean [49], and orchids [50]. SSR markers have also been used for genetic diversity analysis of introduced soybean genotypes. These included reports by Lestari *et al*. [51] on 27 introduced soybean accessions analyzed with 15 SSR markers, Terryana *et al*. [52] on 48 introduced soybean accessions analyzed with 15 SSR markers, and Nugroho *et al*. [19] on 35 introduced soybean genotypes originated from various countries and were analyzed with 15 SSR markers.

Some of the introduced soybean accessions used in this study previously underwent molecular characterization using SSR markers and morphological characterization using secondary data from the United States Department of Agriculture (USDA) database [19, 52]. However, the use of SSR marker types was not described in previous studies [19, 52], and such data, along with morphological data derived from planting outcomes in Indonesia, is an important and new source of information for soybean breeding programs. Thus, the aim of this study was to analyze the genetic diversity of 53 Chinese introduced soybean accessions through morphological and molecular characterization approaches.

2. Materials and Methods

2.1 Study location

The morphological characterization research was conducted at the Cibalagung Experimental Field, Bogor, West Java, Indonesia (250 m above sea level) in 2015. SSR marker analysis was conducted at the Molecular Biology Laboratory, Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor, West Java, Indonesia, from July to September 2021.

2.2 Genetic materials and SSR markers

The genetic material in this study consisted of 53 introduced soybean accessions from China (Table 1). The 53 introduced soybean accessions from China were selected to initiate new genetic variations that could be used to develop new soybean varieties that were more resistant to biotic stresses such as common diseases and insect pests and were tolerant to abiotic stresses such as drought and salinity, and which offered higher yields under tropical short-day conditions. Genomic DNA of each accession was used for SSR markers analysis. The characteristics of the SSR markers used in this study are presented in Table 2.

Table 1. Characteristics of the 53 introduction soybean accessions from GenBank collection, ICABIOGRAD (www.ars-grin.gov)

No	Accession Code	Name of Accession	Origin	Type of Growth	Maturity Group*
1	-	China cult-39	China	-	-
2	-	China cult-38	China	-	-
3	-	China cult-34	China	-	-
4	-	China cult-31	China	-	-
5	-	China cult-32	China	-	-
6	-	China cult-28	China	-	-
7	-	China cult-25	China	-	-
8	PI 092573	7768	Jilin	Indeterminate	Group II
9	PI 079737	N2A	Heilongjiang	Determinate	Group II
10	-	China cult-52	China	-	-
11	-	China cult-60	China	-	-
12	-	China cult-41	China	-	-
13	-	China cult-40	China	-	-
14	PI 088302-2	5691	Liaoning	Indeterminate	Group IV
15	PI 602502	Xiong yue xiao huang dou	China	Determinate	Group III
16	PI 567589	Wan dou li da dou	Shandong	Indeterminate	Group III
17	PI 072232	Wong tau	Jiangxi	Determinate	Group III
18	PI 578499	Lu yue bai	China	Determinate	Group II
19	PI 407721	Muim bao jing	Heilongjiang	Indeterminate	Group II
20	PI 291272	Unknown 2	Heilongjiang	Indeterminate	Group I
21	PI 430620	Hou tzu mao	China	Indeterminate	Group IV
22	PI 587991	Liu yue huang	Sichuan	Determinate	Group III
23	PI 587977	Xiao huang dou	Sichuan	Semi-determinate	Group III
24	PI 567361	Lu fang huang dou	Ningxia	Indeterminate	Group III
25	PI 567359	Hua mei dou	Ningxia	Indeterminate	Group III
26	-	China cult-53	China	-	-
27	-	China cult-55	China	-	-
28	-	China cult-60	China	-	-
29	PI 291312	Unknown 3	Heilongjiang	Indeterminate	Group 0
30	PI 070241	8079	Jilin	Determinate	Group I
31	PI 069501	6946	Jilin	Indeterminate	Group II
32	PI 069992	6790	Jilin	Indeterminate	Group II
33	PI 072341	8969	Liaoning	Determinate	Group II
34	PI 092734	7929	Jilin	Determinate	Group II
35	PI 567302	He se huang dou	Gansu	Indeterminate	Group II
36	PI 567525	Cao qing huang dou	Shandong	Semi-determinate	Group II
37	PI 567537	Gu li hun	Shandong	Semi-determinate	Group II
38	PI 567504	Tu er dun	Hebei	Determinate	Group III
39	PI 171429	An yang black	Henan	Determinate	Group IV
40	PI 430595	58-161	China	Indeterminate	Group IV
41	PI 567318	Hua lai dou	Gansu	Determinate	Group IV
42	PI 567368	Xi hi huang dou	Ningxia	Indeterminate	Group IV
43	PI 567476	Yu ci huang	Shanxi	Indeterminate	Group IV
44	PI 567488 A	Di liu huang dou No.2	Hebei	Indeterminate	Group IV
45	PI 567490	Er huang dou	Hebei	Indeterminate	Group IV
46	PI 567571	Ping ding huang	Shandong	Semi-determinate	Group IV
47	PI 567636	Min quan ba yue zha	Henan	Indeterminate	Group IV

Table 1. Characteristics of the 53 introduction soybean accessions from GenBank collection, ICABIOGRAD (www.ars-grin.gov) (continued)

No	Accession Code	Name of Accession	Origin	Type of Growth	Maturity Group*
48	PI 567769	Tong shan da mian tao	Jiangsu	Indeterminate	Group IV
49	PI 602991	Ni jiao qi do hei dou	Shandong	Determinate	Group IV
50	PI 567343	Ma huang dou	Gansu	Indeterminate	Group V
51	PI 567402	Shi yue han	Shaanxi	Determinate	Group V
52	PI 567634	Mi yang niu mao huang	Henan	Determinate	Group V
53	PI 567657	Tang he huang dou	Henan	Determinate	Group V

Remarks: *Maturity group: 0 = early; X = late

Table 2. Characteristics of the SSR markers used in this study [53, 54]

SSR markers	Chrom	Type of repetition	Primer sequence (5' → 3')	PCR 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: CGCGATCATGTCTCTG R: GGGAGTTGGTGTTTTCTTGTG	226
Satt194	4	(ATT)4gag taaatag(TA)5	F: GGGCCCAACTGATATTTAATTGTAA R: GCGCTTTGTGTTCCGATTTTGAT	246
Satt197	11	(ATT)20	F: CACTGCTTTTTCCCCTCTCT R: AAGATACCCCCAACATTATTTGTAA	173
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
Sat_140	4	(AT)28	F: GCGCCATGAAATTATTTGGCAAGTATT R: GCGGTTGAAGAATGGAAACTAAAAATG	205

Remarks: SSR: Simple Sequence Repeats; Chrom: Chromosom; PCR: Polymerase Chain Reaction; bp = base pair; F: forward; R: reverse

2.3 Procedures

2.3.1 Experimental field

The research was designed using a randomized block design with three replications. Each accession was planted in a small plot of 3 m x 2 m. The planting distance used was 40 cm x 15 cm with 2 plants per hole. The soil was cultivated precisely following the soil processing protocol for soybean adaptation tests [55]. Before land mapping, the soil was fertilized with manure (3 tons/ha). Fertilization was carried out at 12 days after planting with 50 kg urea, 100 kg SP-36, and 100 kg KCl per hectare. Pest and disease control were carried out once a week. Weed control was intensively carried out as needed in the field [55].

2.3.2 Identifying plant morphology

Observation of flower color was carried out during the R1 phase while the flowering time was observed when 50% of the population had flowered. Determination of the maturity time was carried out in the R8 phase when 95% of pods had reached maturity which was indicated by the color of the pods having changed to yellow-brown and the leaves falling. Observation of seed color, hilum color, hair color, plant height, pod number, branch number was carried out after the R8 phase when the plants were harvested. Observation of 100-seed weight was carried out by counting 100 seeds for each sample and then weighing the dry seeds with 12% moisture content. Determination yield/plant was done by weighing the number of seeds from each plant when the seeds were dry with 12% moisture content [56, 57].

2.3.3 Isolation, qualitative and quantitative test of genomic DNA

Genomic DNA was isolated from 0.5 g of young leaf samples of each accession using the Doyle and Doyle method [58], modified by adding 2% (w/v) PVP. The resulting DNA pellet was dissolved in 100 µL of TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA) and 2 µL of 10 mg/mL RNAse (Invitrogen, USA) and incubated at 37°C for 1 h.

The qualitative test of genomic DNA was performed by electrophoresis on a 1% agarose gel. The electrophoresis results were then observed under UV light using a UV Transilluminator (UVP, UK). The quantitative test of genomic DNA for each accession was performed using a NanoDrop 2000 Spectrophotometer (Thermo Scientific™, USA).

2.3.4 PCR analysis and electrophoresis

PCR analysis using 17 SSR markers was carried out on the genomic DNA of each accession tested (Table 2). The genomic DNA of each accession was amplified in a total reaction volume of 10 µL, consisting of 2 µL of 20 ng DNA template, 5 µL of Kapa2G Fast Ready Mix (KAPA Biosystem, USA), 0.5 µL of each 10 µM forward and reverse primer, and 2 µL of sterile ddH2O. The PCR protocol, based on a studied conducted by Tasma *et al*. [12, 35], included initial denaturation at 95°C for 5 min, DNA amplification for 35 cycles with denaturation at 94°C for 30 s, annealing of primers at 55°C for 1 min, extension of DNA at 72°C for 1 min, post-extension of DNA at 60°C for 15 min, and DNA incubation at 10°C for 4 min.

The PCR results were then subjected to electrophoresis using an 8% polyacrylamide gel in a vertical electrophoresis tank that had been filled with 1x Tris Borate EDTA (TBE) buffer for 115 min at 90 volts. Visualization of DNA bands was performed using ethidium bromide staining on a UV Transilluminator Gel Doc (Bio Rad, California, USA).

2.4 Data analysis

2.4.1 Morphological data analysis

The morphological characteristics observed were flowering time, maturity time, flower color, seed color, hilum color, pubescent color, plant height, branch number/plant, pod number/plant, 100-seed weight, and seed yield/plant. Before analysis, qualitative morphological data were converted into quantitative data. Pearson correlation is the most widely used correlation statistic to measure the degree of the relationship between linearly related variables, and Pearson correlation was conducted in this study using R Studio software. PCA and PCoA were performed on all morphological data, and the analysis was conducted using R Studio software [59].

2.4.2 Molecular data analysis

Scoring of DNA band patterns was performed using GelAnalyzer software v.2010a [60]. The scoring data were analyzed using the sequential agglomerative hierarchical and nested (SAHN) - unweighted pair group method with arithmetic (UPGMA) program on NTSYS-pc software version 2.1 [61] to obtain a dendrogram of relationships among accessions. Other data analysis was carried out using PowerMarker V3.25 software [62] to obtain statistics on polymorphism information content (PIC), major allele frequency, genetic diversity, and heterozygosity of each SSR marker.

3. Results and Discussion

3.1 Morphological analysis

Diversity plays a crucial role in plant breeding programs [15]. The assessment of the diversity of a particular crop species creates a foundational data source for selecting parental lines in a plant breeding program. Crossing genotypes from the same cluster is not favorable because it does not result in desirable segregates. When genotypes with similar genetic characteristics are grouped together in the same cluster, it indicates limited diversity [63]. Conversely, genotypes with greater genetic distance, represented by diverged clusters, signify higher diversity between the clusters. The approach to determine diversity within a population can be observed through morphological and molecular characteristics.

The morphological characteristics of soybean accessions introduced from China are presented in Table 3. The flowering time of introduced accessions ranged from 26-34 days after planting (DAP) and maturity time ranged from 67-80 DAP. There were 22 introduced accessions with purple flowers and 31 accessions with white flowers. There were 44 accessions with yellow seed color, yellowish green (1 accession), green (1 accession), brown (3 accessions), dark brown (1 accession), and black (3 accessions). The variation in hilum color included gray, yellow, light brown, dark brown, black, and brown. The variation in pubescent color included gray and brown in balanced proportions. Plant height ranged from 24-74 cm, branch number/plant ranged from 0-4, pod number/plant ranged from 16-58. The weight of 100 seeds ranged from 6.75-23.38 g and seed yield/plant ranged from 2.86-13.26 g.

Table 3. Morphological characteristics of 53 Chinese soybean introduction accessions at the Cibalagung Experimental Station, Bogor, West Java

No	Name of Accession	FT (dap)	MT (dap)	FC	SC	HC	PC	PH (cm)	NBP	NPP	100W (g)	SYP (g)
1	China cult-39	28	76	W	Y	Y	GY	53.0	2	29	16.37	9.79
2	China cult-38	28	80	W	Y	DB	GY	45.0	1	32	16.41	6.34
3	China cult-34	28	80	W	Y	LB	BR	49.0	1	36	17.62	10.08
4	China cult-31	26	75	W	Y	Y	GY	52.0	1	28	15.47	8.00
5	China cult-32	26	75	P	Y	Y	BR	39.0	1	23	15.96	6.33
6	China cult-28	26	80	P	Y	Y	BR	43.0	0	25	18.24	8.56
7	China cult-25	28	80	P	Y	Y	GY	62.5	0	37	17.91	6.92
8	7768	28	70	P	Y	Y	GY	43.0	2	32	12.44	6.60
9	N2A	28	76	P	Y	BL	BR	47.5	2	30	14.65	6.98
10	China cult-52	28	75	W	Y	Y	BR	47.5	1	26	16.76	9.68
11	China cult-60	28	76	W	Y	Y	BR	45.0	1	25	16.97	6.19
12	China cult-41	28	76	W	Y	Y	GY	45.5	1	24	15.14	6.62
13	China cult-40	28	76	W	Y	BR	GY	48.0	1	28	13.80	8.75
14	5691	34	80	P	Y	GY	GY	55.0	3	28	16.92	2.86
15	Xiong yue xiao huang dou	34	76	W	Y	BL	GY	56.0	3	58	12.30	11.32
16	Wan dou li da dou	34	70	W	Y	BR	GY	41.0	3	18	17.59	6.88
17	Wong tau	32	70	P	Y	BL	BR	50.0	4	27	11.47	6.35
18	Lu yue bai	30	76	P	GR	BL	BR	37.5	3	27	17.79	8.59
19	Muim bao jing	30	76	W	Y	BR	BR	53.0	2	24	18.81	7.31
20	Unknown 2	32	76	P	BL	BL	BR	60.0	3	49	9.99	7.33
21	Hou tzu mao	32	76	P	YG	BR	GY	42.5	2	34	15.31	9.10
22	Liu yue huang	32	67	W	Y	DB	BR	50.5	3	32	11.73	7.59
23	Xiao huang dou	32	67	W	Y	BR	BR	59.5	3	28	11.66	7.59
24	Lu fang huang dou	28	77	W	Y	DB	BR	51.5	4	31	9.03	3.21
25	Hua mei dou	28	79	W	DB	BR	BR	57.0	4	26	17.67	4.49
26	China cult-53	28	75	P	Y	Y	BR	37.0	1	25	20.95	6.43
27	China cult-55	28	75	W	Y	Y	GY	47.0	1	24	18.70	6.54
28	China cult-60	28	75	W	Y	Y	GY	43.0	0	20	18.25	7.08

Remarks: dap = days after planting, FT = flowering time, MT = maturity time, = FC = flower color, SC = seed color, HC = hilum color, PC = pubescent color, PH = plant height, NBP = branch number/plant, NPP = pod number/plant, 100W = 100-seed weight, SYP = seed yield/plant, W = white, P = purple, Y = yellow, GR = green, BL = black, YG = yellowish-green, BR = brown, DB = dark brown, LB = light brown, GY = Gray

Table 3. Morphological characteristics of 53 Chinese soybean introduction accessions at the Cibalagung Experimental Station, Bogor, West Java (continued)

No	Name of Accession	FT (dap)	MT (dap)	FC	SC	HC	PC	PH (cm)	NBP	NPP	100W (g)	SYP (g)
29	Unknown 3	28	75	P	Y	Y	BR	45.5	1	28	19.52	7.08
30	8079	28	75	P	Y	BR	GY	42.0	2	34	14.00	10.58
31	6946	28	75	W	Y	BR	GY	62.0	2	28	15.55	7.69
32	6790	28	75	W	Y	BR	BR	53.5	3	38	16.82	9.75
33	8969	28	75	W	Y	LB	GY	36.0	1	27	14.19	8.01
34	7929	28	75	P	Y	BR	GY	61.0	2	30	15.97	7.63
35	He se huang dou	28	70	P	BR	BR	BR	44.5	2	34	11.43	6.30
36	Cao qing huang dou	30	70	P	Y	BL	BR	67.5	3	31	12.56	9.29
37	Gu li hun	30	74	P	Y	BR	GY	52.5	3	40	14.47	10.29
38	Tu er dun	28	78	W	Y	LB	GY	27.0	3	28	20.68	6.37
39	An yang black	28	78	W	BL	BL	BR	39.0	1	20	11.20	4.90
40	58-161	28	74	W	Y	LB	BR	39.0	3	29	23.38	12.36
41	Hua lai dou	30	74	W	BR	BR	BR	24.0	2	28	8.57	6.08
42	Xi hi huang dou	30	80	P	Y	BL	BR	64.5	4	35	10.50	5.33
43	Yu ci huang	30	74	P	Y	BR	BR	70.0	2	34	9.98	5.53
44	Di liu huang dou No.2	30	80	W	Y	DB	BR	58.0	2	32	14.26	7.82
45	Er huang dou	30	80	P	Y	DB	BR	74.0	3	42	8.30	5.90
46	Ping ding huang	26	80	W	Y	BL	GY	32.5	2	21	20.10	8.13
47	Min quan ba yue zha	30	79	W	Y	DB	GY	71.5	2	38	9.38	6.31
48	Tong shan da mian tao	30	79	W	Y	DB	GY	71.0	2	36	17.96	10.06
49	Ni jiao qi do hei dou	30	80	W	BL	BL	BR	39.0	1	16	17.29	6.21
50	Ma huang dou	30	74	P	BR	BR	GY	70.5	3	56	6.75	6.34
51	Shi yue han	30	70	P	Y	LB	GY	47.0	4	39	11.50	8.07
52	Mi yang niu mao huang	30	70	W	Y	DB	BR	52.5	3	34	13.49	10.60
53	Tang he huang dou	30	78	W	Y	DB	BR	45.0	2	34	14.75	13.26
	Average	29.17	75.51					49.99	1.84	30.66	14.87	7.61

Remarks: dap = days after planting, FT = flowering time, MT = maturity time, = FC = flower color, SC = seed color, HC = hilum color, PC = pubescent color, PH = plant height, NBP = branch number/plant, NPP = pod number/plant, 100W = 100-seed weight, SYP = seed yield/plant, W = white, P = purple, Y = yellow, GR = green, BL = black, YG = yellowish-green, BR = brown, DB = dark brown, LB = light brown, GY = Gray

Based on the morphological characteristics, flowering and maturity time Table 3, all soybean accessions introduced from China used in this study were able to be classified into the early-maturing group according to the criteria established by Rahajeng and Adie [64]. The maturity group was categorized into 4 groups [64]: early-maturing (<79 days), intermediate (80-85 days), medium (86-90 days), and late-maturing (>90 days). However, based on other morphological characteristics (Table 3), there was diversity observed in qualitative traits such as flower color, seed color, hilum color, and pubescent color. Quantitative traits, such as yield and yield components also exhibited diversity.

The diversity observed in qualitative traits is primarily attributed to genetic factors, whereas the diversity observed in quantitative morphological traits is influenced not only by genetic factors but also by environmental factors [65]. Therefore, a more in-depth evaluation of quantitative traits is necessary as environmental factors significantly impact the expressed traits. Thus, it is important to align the observations of morphological traits with molecular data to ensure that selected parents in plant breeding programs have a significant genetic distance. This is expected to promote the emergence of superior genotypes.

Pearson correlation matrix values for the 11 morphological characteristics indicated that not all characteristics resulted in a significant positive correlation (Table 4). Plant height and pod number/plant showed a significant positive correlation, with a matrix correlation value of 0.54 at $\alpha = 0.01$ and 0.05. Other morphological characteristics that showed significant positive correlations were branch number/plant and flowering time (0.53), branch number/plant and hilum color (0.43), pod number/plant and branch number/plant (0.38), hilum color and seed color (0.38), pod number/plant and flowering time (0.35), hilum color and flowering time (0.32), pod number/plant and pubescent color (0.29), pubescent color and seed color (0.28), and plant height and flowering time (0.28).

The interconnections between different morphological characteristics can be examined through Pearson correlation analysis (Table 4), which assesses the relationships and associations among observed morphological traits. High and significantly positive values of the Pearson correlation matrix indicate a strong correlation among morphological characteristics [66]. The significant positive correlations obtained in this research facilitate the optimization of genetic improvement of the introduced soybean accessions through crossbreeding with desired target traits to obtain superior genotypes.

Table 4. Pearson correlation matrix values of 11 morphological characteristics among 53 soybean accessions introduced from China

Char	FT	MT	FC	SC	HC	PC	PH	NBP	NPP	100W
MT	-0.27									
FC	0.09	-0.11								
SC	0.09	0.09	0.03							
HC	0.32[b]	-0.19	0.08	0.38[b]						
PC	-0.05	0.02	0.11	0.28[b]	0.12					
PH	0.28[b]	0.06	0.19	-0.11	0.17	-0.02				
NBP	0.53[ab]	-0.3[b]	0.14	0.07	0.43[ab]	0.12	0.27[b]			
NPP	0.35[b]	-0.01	0.29[b]	0.03	0.20	-0.13	0.54[ab]	0.38[ab]		
100W	-0.35[b]	0.26	-0.22	-0.27	-0.37[ab]	-0.07	-0.43[ab]	-0.38[ab]	-0.52[ab]	
SYP	-0.01	-0.14	-0.17	-0.29[b]	0.05	-0.07	-0.05	-0.05	0.26	0.26

Remarks: Char = characteristics, FT = flowering time, MT = maturity time, = FC = flower color, SC = seed color, HC = hilum color, PC = pubescent color, PH = plant height, NBP = branch number/plant, NPP = pod number/plant, 100W = 100-seed weight, SYP = seed yield/plant, [a] = significant correlation $\alpha = 0.01$, [b] = significant correlation at $\alpha = 0.05$

PCA in this study enabled the reduction of morphological characteristics into 4 principal components with eigenvalue >1, which explained a total variance of 64.67% in 53 accessions (Table 5). The first principal component with an eigenvalue of 3.08 explains 27.98% of the variance, including flower color, hilum color, plant height, branch number/plant, and pod number/plant. The second principal component with an eigenvalue of 1.66 explains 15.09% of the variance, including the seed color and pubescent color. The third principal component with an eigenvalue of 1.33 accounted for 12.09% of the variance, including maturity time and plant height. The fourth principal component with an eigenvalue of 1.05 accounted for 9.52% of the variance, including maturity time.

Table 5. Principal component analysis (PCA) of morphological characteristics of 53 soybean accessions introduced from China

Characteristics	PC 1	PC 2	PC 3	PC 4
Flowering time	**0.67**	-0.12	-0.25	-0.02
Maturity time	-0.33	0.14	**0.63**	**0.53**
Flower color	0.36	0.05	0.37	-0.52
Seed color	0.25	**0.76**	-0.01	0.31
Hilum color	**0.60**	0.28	-0.34	0.32
Pubescent color	0.09	**0.57**	-0.08	0.06
Plant height	**0.59**	-0.29	**0.50**	0.13
Branch number/plant	**0.73**	-0.01	-0.28	-0.07
Pod number/plant	**0.69**	-0.40	0.30	0.26
100-seed weight	-0.77	-0.15	-0.18	0.09
Seed yield/plant	-0.08	-0.60	-0.41	0.43
Eigenvalue	3.08	1.66	1.33	1.05
Variance (%)	27.98	15.09	12.09	9.52
Cumulative (%)	27.98	43.06	55.16	64.67

Remarks: PC = principal components, the bold numbers contribute to the variance.

PCA is a commonly used analysis for reducing variables and genotypes in a population [67]. The genetic diversity within a population can be assessed through PCA based on observed morphological characteristics. Table 5 shows that 4 principle components were identified which accounted for the genetic diversity of the introduced soybean accessions from China based on eigenvalue scores. The eigenvalue scores are used to determine the number of variables that should be retained. Eigenvalue > 1 were capable of explaining the genetic diversity [68]. Each principle component contributes to the morphological characteristics that generate the genetic diversity. The contribution of each morphological characteristic is determined by the value of the principle component. Morphological characteristics with a principle component value >0.5 were considered to have significant contribution [69]. There are slight differences between the findings of this study and previous studies reported by Terryana *et al*. [52], Nugroho *et al*. [19], and Lestari *et al*. [51], which relied on secondary data. In this study, a total genetic diversity value of 64.67% was observed with 4 principal components, whereas the previous studies reported a total genetic diversity of 46.92% with 2 principal components. This study provides additional information, making it more comprehensive due to the planting location being in Indonesia.

The relative positions of the 53 soybean accessions introduced from China based on morphological characteristics in a two-dimensional space obtained through PCoA can provide opportunities to enhance plant selection activities (Figure 1). The 53 soybean accessions introduced from China tended to spread out and overlap in four quadrants, indicating similarities in morphological characteristics among the accessions. For example, I16 (wan dou li da dou) and

I32 (6790) fall in the same quadrant, indicating similar morphological characteristics based on observations (Table 3) such as flower color, seed color, and hilum color. Additionally, both accessions have the same type of growth, which is indeterminate (Table 1). This suggests that accessions within the same quadrant are not recommended to be used as parents in breeding programs due to their close genetic diversity. There are several introduced soybean accessions that do not overlap and are located in different quadrants, i.e., I15 (Xiong yue xiao huang dou), I20 (Unknown 2), and I50 (ma huang dou), indicating morphological differences. These accessions have a higher chance of being considered as parental candidates in developing new superior varieties.

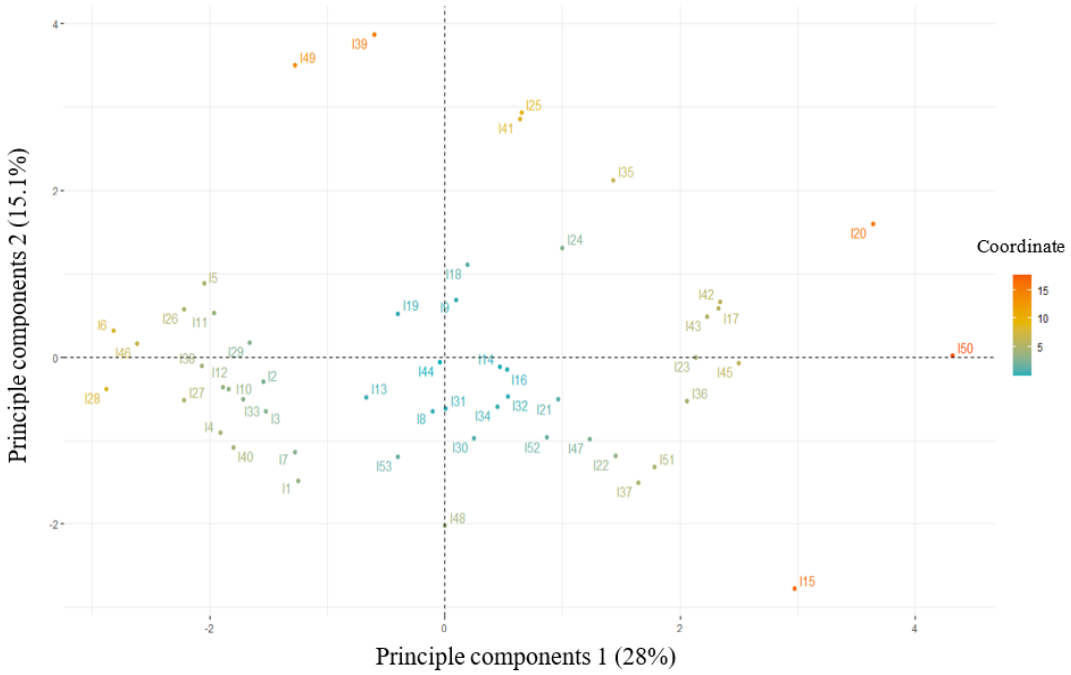

Figure 1. The relative positions of the 53 soybean accessions introduced from China based on morphological characteristics through Principal Coordinate Analysis (PCoA). Number 1-53 are introduced soybean accessions with their characteristics as shown in Table 1.

PCoA is used to assess proximity among individuals based on similarities in traits [70]. The PcoA revealed that a majority of the introduced soybean accessions from China overlapped in the same quadrants (Figure 1). According to Lestari *et al*. [51], the overlapping of soybean accessions in the same quadrants is attributed to similarities in morphological characteristics such as flower color, pod, seed, hilum color, growth type, and others. Consequently, accessions that overlap in the same quadrant cannot be used as parent sources for crossing due to their close genetic relatedness.

3.2 Molecular analysis

All SSR markers were able to demonstrate polymorphic band patterns in the 53 soybean accessions introduced from China (Table 6). A total of 772 alleles were detected, with a range of 29-62 alleles per locus and an average of 45 alleles per locus. The average frequency of the main allele was 10%, with the lowest value being 4% (Satt191) and the highest value being 26% (Satt063). The percentage of genetic diversity, indicating the level of genetic variation within a population, ranged from 90% (Satt063) to 98% (Satt191, Satt194, Satt197, and Satt431), with an average of 96%. All SSR markers could detect heterozygous alleles, with values ranging from 0.38 (Satt038) to 1.00 (Satt045, Satt191, Satt431, and Satt646). The polymorphic information content (PIC) ranged from 0.89 (Satt063) to 0.98 (Satt191, Satt194, Satt197, and Satt431), with an average PIC value of 0.96.

Table 6. The characteristics of 17 SSR markers used to analyze genetic diversity of 53 introduced soybean accessions from China

Markers	Allele Number	Allele Size (bp)	Main Allele Frequency	Gene Diversity	Heterozigosity	PIC
satt002	32	142-186	0.13	0.94	0.98	0.93
satt009	45	151-270	0.10	0.96	0.98	0.96
satt030	46	156-220	0.07	0.97	0.83	0.97
satt038	37	157-231	0.12	0.95	0.38	0.95
satt045	42	138-202	0.09	0.96	1.00	0.96
satt063	29	119-193	0.26	0.90	0.64	0.89
satt114	30	85-134	0.12	0.95	0.75	0.94
satt147	47	163-252	0.08	0.97	0.98	0.97
satt191	62	188-297	0.04	0.98	1.00	0.98
satt194	60	216-315	0.08	0.98	0.96	0.98
satt197	61	143-247	0.05	0.98	0.98	0.98
satt308	46	134-203	0.07	0.97	0.96	0.97
satt431	62	195-294	0.04	0.98	1.00	0.98
satt463	45	135-284	0.15	0.95	0.62	0.95
satt607	43	222-326	0.09	0.96	0.47	0.96
satt646	44	182-251	0.07	0.97	1.00	0.97
sat_140	41	190-264	0.07	0.97	0.58	0.97
Total	772					
Average	45		0.10	0.96	0.83	0.96

Remarks: PIC = polymorphism information content

Genetic diversity analysis in this studies revealed the detection of 772 alleles (Table 6). It was a higher number of alleles than in the study conducted by Terryana *et al*. [52], in which 226 alleles in 48 soybean accessions using 15 SSR markers were detected. Additionally, the study by Tasma *et al*. [12] reported a lower number of alleles, with 316 alleles detected in 29 soybean genotypes using 27 SSR markers. The larger number of soybean accessions and molecular markers used in this study resulted in a higher number of detected alleles. The number of alleles and the values of genetic diversity derived from SSR marker analysis are interconnected. In this study, a higher number of alleles led to a higher genetic diversity value. This is supported by previous research conducted by Asadi *et al*. [71], who reported that the lowest number of alleles (9

alleles) resulted in a genetic diversity value of 79%, while the highest number of alleles (28 alleles) resulted in a genetic diversity value of 96%.

The genetic diversity within a population can be assessed through heterozygosity values [72]. Measurement of heterozygosity can provide a comparison of the number of individual heterozygous alleles within a population. All SSR markers using in this study indicated heterozygous alleles with an average value of 0.83 (Table 6). The markers satt045, satt191, satt431, and satt646 exhibited a heterozygosity value of 1, indicating that the alleles were 100% heterozygous alleles.

PIC value and genetic diversity were fond to be positively correlated [51, 73, 74]. This is consistent with the findings of this study, the marker Satt063, with the lowest genetic diversity value of 90%, has a PIC value of 0.89 (Table 6). On the other hand, the markers Satt191, Satt194, Satt197, and Satt431, with the highest genetic diversity value of 98%, have PIC values of 0.98 (Table 6). According to Tasma and Arumsari [75], the PIC value and the number of alleles depend on the characteristics of the SSR markers and the diversity of the tested accessions. A higher PIC value indicates more informative molecular markers. The PIC value is essential for selecting markers that can distinguish one accession from another. The level of polymorphism can also be determined by the number of alleles generated by each marker. Markers that produce fewer alleles have a lower ability to distinguish the tested samples [76].

Phylogenetic analysis of the 53 soybean accessions introduced from China based on 17 SSR markers in this study resulted in a genetic similarity level of 82% (Figure 2; Table 7). Two main clusters were identified based on the genetic similarity level. Cluster I consisted of 49 accessions, while Cluster II consisted of 4 accessions (China cult-53, China cult-55, China cult-60, and Unknown 3). Several accessions clustered together based on growth type (indeterminate) included Unknown 2, Lu fang huang dou, and He se huang dou in Cluster I. There were also accessions that clustered in Cluster I based on their origin, such as accessions 8079, 6946, 6790, and 7929 from Jilin province, as well as Yu ci huang and Shi yue han from Shanxi province. Additionally, there were soybean accessions that clustered in Cluster I but were of unknown growth type and maturity group such as China cult-38, China cult-32, China cult-28, China cult-52, China cult-40, China cult-31, and China cult-41 (Figure 2).

There were two introduced soybean accessions with the highest genetic similarity value (Table 7), which indicated a very close relationship. These accessions were 6946 and 6790, with a genetic similarity value of 0.96. This genetic similarity value indicated a 96% genetic similarity between the two accessions, with a difference of 4%. Additionally, there were soybean accessions that had a distant relationship, e.g., China cult-55 and Mi yang niu mao huang. The genetic similarity value between these two accessions was 0.78, which meant there was a 78% genetic similarity, or a difference of 22% (Table 7).

Clustering in phylogenetic analysis can be used for parent selection in breeding programs. In this study, the phylogenetic analysis resulted in two main clusters with a genetic similarity of 82% (Figure 2, Table 7). This was consistent with previous studies that reported genetic similarities of 75% with two main clusters [51], 82% with two main clusters [19], and 84% with three main clusters [52]. Accessions within the same cluster show high genetic similarity.

According to Hossain *et al*. [73], genetic similarity values can be used to determine the level of relatedness between analyzed genotypes. The grouping of several accessions in Cluster I was based on growth type, province of origin, and accessions with unknown growth type and maturity group. The low genetic similarity value in parent accessions can result in high genetic diversity for the next generation progenies. Accessions with a low genetic similarity value of 78% (Table 7), such as China cult-55 and Mi yang niu mao huang, can be selected as parents for crossbreeding in the development of new superior varieties. Selection of two accessions as parents was carried out to avoid the occurrence of inbreeding depression. Soybean is classified as a self-

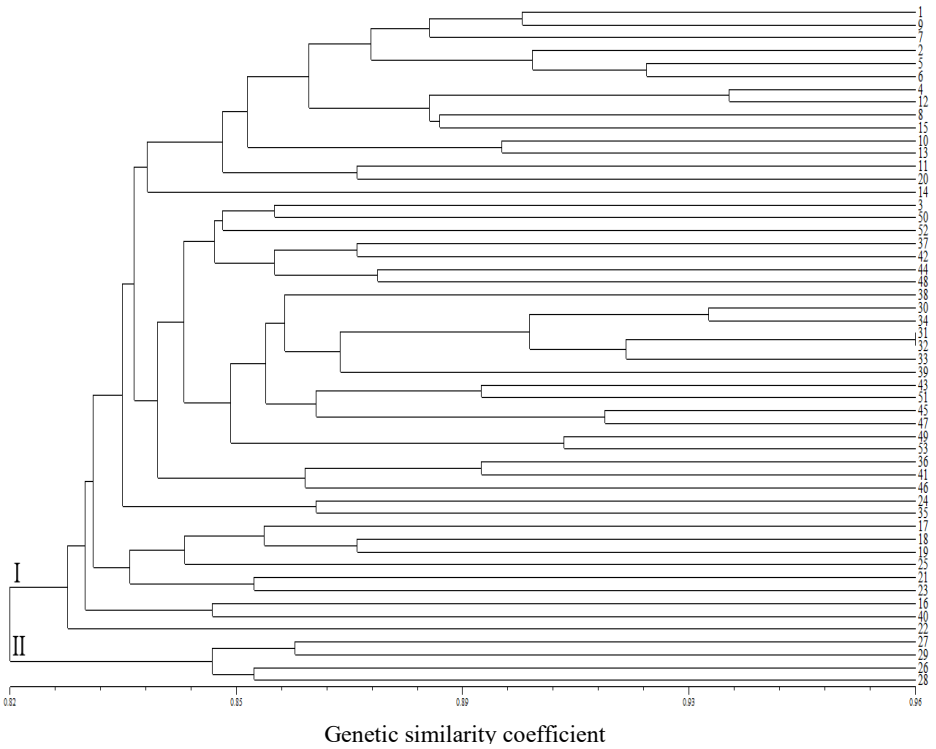

Figure 2. Dendrogram of 53 introduced soybean accessions from China based on 17 SSR markers. 1-53 = number of different soybean accessions introduced from China as listed in Table 1.

pollinating crop. Continuous self-pollination in soybeans will produce homozygous alleles from parents with high genetic similarity. If a homozygous gene has recessive alleles that carry bad traits, then inbreeding depression will occur [77].

The results of genetic diversity analysis based on morphological characters using PCoA and molecular markers using phylogenetic analysis showed consistency in grouping the introduced soybean accessions from China. This consistency was demonstrated by the clustering of four introduced soybean accessions (8079, 6946, 6790, and 7929) in the same cluster based on the phylogenetic analysis (Figure 2). These accessions also clustered in the same quadrant based on PCoA (Figure 1). Furthermore, based on the phylogenetic analysis, two accessions showed low genetic similarity of 78% (Figure 2, Table 7), i.e., China cult-55 and Mi yang niu mao huang. Consistently, according to the PCoA analysis (Figure 1), these two accessions were positioned in different quadrants. Therefore, genetic diversity analysis should be performed by integrating analyses based on morphological characters and molecular markers to select parents for crossbreeding and develop new superior varieties.

Table 7. Genetic similarity matrix of 53 soybean accessions introduced from China obtained from the analysis using 17 SSR markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
2	0.88																																																				
3	0.85	0.86																																																			
4	0.88	0.86	0.86																																																		
5	0.87	0.89	0.86	0.86																																																
6	0.86	0.90	0.85	0.88	0.91																																															
7	0.88	0.85	0.81	0.85	0.88	0.88																																															
8	0.84	0.86	0.86	0.88	0.84	0.87	0.84																																													
9	0.89	0.86	0.81	0.87	0.89	0.85	0.88	0.84																																												
10	0.85	0.88	0.84	0.83	0.87	0.87	0.85	0.85	0.86																																											
11	0.86	0.83	0.84	0.86	0.82	0.83	0.84	0.84	0.84	0.83																																									
12	0.88	0.87	0.85	0.93	0.85	0.87	0.85	0.88	0.87	0.84	0.89																																								
13	0.85	0.85	0.82	0.82	0.85	0.86	0.84	0.85	0.85	0.89	0.83	0.83																																							
14	0.83	0.85	0.81	0.83	0.81	0.84	0.83	0.83	0.84	0.82	0.83	0.84	0.84																																							
15	0.85	0.88	0.85	0.87	0.86	0.88	0.82	0.88	0.85	0.86	0.87	0.88	0.83	0.84																																						
16	0.83	0.83	0.82	0.86	0.83	0.83	0.84	0.87	0.84	0.83	0.85	0.86	0.84	0.83	0.84																																						
17	0.84	0.83	0.82	0.83	0.81	0.80	0.82	0.82	0.84	0.80	0.85	0.83	0.81	0.82	0.82	0.82																																						
18	0.83	0.85	0.84	0.83	0.83	0.80	0.81	0.81	0.83	0.83	0.82	0.83	0.85	0.81	0.82	0.82	0.84																																					
19	0.86	0.84	0.83	0.82	0.82	0.81	0.80	0.82	0.83	0.83	0.80	0.81	0.84	0.81	0.81	0.81	0.86	0.87																																					
20	0.89	0.85	0.84	0.83	0.82	0.82	0.82	0.85	0.84	0.84	0.87	0.86	0.86	0.83	0.84	0.84	0.84	0.84	0.87																																				
21	0.82	0.81	0.82	0.82	0.79	0.79	0.80	0.82	0.83	0.79	0.81	0.82	0.79	0.80	0.81	0.80	0.84	0.83	0.84	0.83																																			
22	0.85	0.81	0.81	0.84	0.82	0.79	0.83	0.84	0.83	0.83	0.84	0.83	0.83	0.81	0.83	0.83	0.83	0.81	0.81	0.83	0.84																																		
23	0.84	0.83	0.84	0.82	0.81	0.83	0.82	0.81	0.83	0.82	0.81	0.81	0.81	0.82	0.83	0.81	0.82	0.83	0.82	0.84	0.85	0.81																																		
24	0.84	0.84	0.83	0.81	0.84	0.83	0.83	0.82	0.83	0.83	0.82	0.83	0.83	0.81	0.83	0.83	0.82	0.83	0.84	0.86	0.82	0.81	0.83																																		
25	0.84	0.86	0.82	0.82	0.82	0.81	0.81	0.82	0.82	0.83	0.80	0.81	0.83	0.80	0.81	0.81	0.83	0.83	0.86	0.84	0.84	0.81	0.81	0.85																																		
26	0.80	0.82	0.81	0.80	0.80	0.80	0.80	0.79	0.80	0.80	0.83	0.79	0.80	0.80	0.82	0.81	0.83	0.82	0.81	0.79	0.81	0.81	0.83	0.80	0.82																																		
27	0.84	0.82	0.80	0.81	0.82	0.79	0.81	0.81	0.83	0.81	0.82	0.79	0.81	0.81	0.81	0.81	0.81	0.81	0.82	0.79	0.82	0.82	0.80	0.81	0.80	0.84																																		
28	0.82	0.82	0.82	0.79	0.79	0.79	0.80	0.80	0.80	0.80	0.81	0.80	0.81	0.80	0.80	0.81	0.82	0.82	0.79	0.82	0.82	0.79	0.83	0.83	0.82	0.85	0.85																																		
29	0.83	0.81	0.82	0.80	0.79	0.79	0.81	0.81	0.81	0.79	0.83	0.80	0.80	0.81	0.79	0.83	0.82	0.82	0.82	0.81	0.80	0.81	0.82	0.81	0.81	0.84	0.86	0.84																																		
30	0.87	0.87	0.85	0.84	0.85	0.84	0.82	0.84	0.89	0.86	0.82	0.84	0.84	0.84	0.84	0.82	0.85	0.84	0.87	0.84	0.83	0.81	0.84	0.83	0.83	0.82	0.83	0.81	0.81																																		
31	0.85	0.83	0.87	0.83	0.81	0.81	0.81	0.85	0.84	0.82	0.81	0.82	0.81	0.80	0.83	0.82	0.84	0.82	0.84	0.85	0.82	0.82	0.83	0.83	0.83	0.84	0.82	0.83	0.83	0.89																																		
32	0.86	0.84	0.87	0.85	0.82	0.82	0.82	0.85	0.86	0.92	0.82	0.84	0.81	0.82	0.82	0.84	0.86	0.82	0.85	0.86	0.83	0.92	0.82	0.83	0.84	0.82	0.83	0.82	0.83	0.92	0.96*																																		
33	0.85	0.86	0.85	0.85	0.84	0.85	0.83	0.87	0.87	0.85	0.82	0.85	0.83	0.81	0.85	0.84	0.83	0.81	0.82	0.85	0.82	0.83	0.82	0.83	0.84	0.81	0.81	0.81	0.81	0.88	0.90	0.92																																		
34	0.87	0.85	0.83	0.84	0.84	0.82	0.82	0.83	0.90	0.82	0.81	0.83	0.82	0.83	0.82	0.82	0.82	0.82	0.85	0.86	0.83	0.82	0.83	0.81	0.84	0.82	0.82	0.82	0.81	0.93	0.88	0.91	0.89																																		
35	0.83	0.84	0.84	0.83	0.81	0.83	0.81	0.83	0.82	0.83	0.83	0.83	0.82	0.80	0.82	0.82	0.82	0.80	0.83	0.84	0.83	0.81	0.81	0.86	0.82	0.82	0.81	0.82	0.82	0.85	0.84	0.85	0.87	0.84																																		
36	0.82	0.80	0.84	0.85	0.80	0.81	0.83	0.85	0.82	0.79	0.86	0.83	0.80	0.83	0.81	0.84	0.83	0.83	0.82	0.83	0.83	0.83	0.80	0.81	0.81	0.83	0.84	0.81	0.84	0.85	0.86	0.89	0.85	0.85	0.85																																		
37	0.84	0.82	0.84	0.82	0.80	0.81	0.80	0.82	0.83	0.83	0.79	0.81	0.83	0.79	0.82	0.81	0.84	0.86	0.82	0.81	0.83	0.82	0.84	0.80	0.82	0.82	0.80	0.82	0.81	0.86	0.86	0.85	0.84	0.85	0.83	0.84																																		
38	0.85	0.85	0.84	0.85	0.85	0.82	0.82	0.82	0.83	0.82	0.82	0.84	0.82	0.80	0.82	0.84	0.84	0.86	0.83	0.84	0.82	0.81	0.81	0.85	0.83	0.80	0.81	0.82	0.81	0.86	0.86	0.86	0.85	0.86	0.84	0.83	0.85																																		
39	0.85	0.84	0.84	0.83	0.84	0.82	0.82	0.83	0.84	0.81	0.82	0.84	0.82	0.81	0.82	0.82	0.82	0.84	0.83	0.84	0.81	0.81	0.82	0.84	0.82	0.83	0.82	0.84	0.83	0.86	0.88	0.88	0.85	0.85	0.84	0.85	0.84	0.86																																		
40	0.83	0.85	0.83	0.82	0.80	0.82	0.80	0.83	0.81	0.82	0.79	0.81	0.84	0.80	0.82	0.84	0.83	0.84	0.83	0.82	0.81	0.81	0.81	0.81	0.82	0.81	0.79	0.80	0.80	0.82	0.82	0.81	0.82	0.82	0.81	0.81	0.84	0.82	0.82																																		
41	0.83	0.81	0.83	0.83	0.81	0.82	0.84	0.83	0.81	0.80	0.84	0.81	0.81	0.83	0.82	0.81	0.80	0.82	0.80	0.84	0.83	0.81	0.81	0.82	0.80	0.82	0.83	0.81	0.82	0.82	0.84	0.84	0.83	0.83	0.84	0.89	0.83	0.86	0.84	0.81																																		
42	0.85	0.87	0.82	0.83	0.83	0.83	0.80	0.83	0.84	0.85	0.81	0.84	0.87	0.84	0.84	0.82	0.81	0.83	0.83	0.84	0.81	0.81	0.82	0.82	0.85	0.81	0.80	0.82	0.81	0.87	0.83	0.82	0.84	0.86	0.83	0.81	0.87	0.86	0.85	0.83	0.83																																		
43	0.85	0.85	0.85	0.85	0.82	0.83	0.81	0.85	0.86	0.84	0.83	0.84	0.84	0.84	0.82	0.84	0.84	0.86	0.84	0.85	0.84	0.83	0.83	0.83	0.84	0.82	0.81	0.81	0.83	0.88	0.86	0.86	0.85	0.87	0.85	0.84	0.85	0.86	0.86	0.84	0.85	0.86																																		
44	0.87	0.84	0.86	0.86	0.84	0.85	0.81	0.88	0.83	0.85	0.81	0.83	0.85	0.82	0.83	0.83	0.81	0.81	0.86	0.86	0.79	0.82	0.80	0.82	0.82	0.79	0.79	0.80	0.82	0.87	0.86	0.85	0.84	0.85	0.83	0.82	0.84	0.84	0.83	0.83	0.83	0.87	0.86																																		
45	0.85	0.85	0.85	0.84	0.81	0.82	0.81	0.83	0.84	0.81	0.81	0.82	0.82	0.82	0.82	0.84	0.82	0.84	0.81	0.84	0.81	0.80	0.83	0.83	0.83	0.82	0.81	0.80	0.83	0.86	0.86	0.86	0.87	0.86	0.85	0.83	0.83	0.88	0.86	0.82	0.84	0.84	0.90	0.82																																		
46	0.83	0.83	0.83	0.82	0.83	0.83	0.84	0.85	0.82	0.81	0.81	0.82	0.82	0.84	0.81	0.82	0.81	0.83	0.81	0.82	0.82	0.82	0.80	0.83	0.81	0.81	0.81	0.82	0.81	0.81	0.82	0.82	0.83	0.82	0.82	0.85	0.81	0.86	0.86	0.82	0.87	0.82	0.85	0.83	0.84																																		
47	0.83	0.84	0.86	0.82	0.83	0.84	0.82	0.86	0.84	0.84	0.80	0.81	0.83	0.83	0.85	0.82	0.81	0.83	0.81	0.80	0.80	0.82	0.84	0.81	0.81	0.82	0.80	0.79	0.80	0.86	0.86	0.84	0.84	0.83	0.81	0.84	0.86	0.83	0.84	0.81	0.82	0.82	0.86	0.84	0.91	0.83																																		
48	0.84	0.85	0.83	0.85	0.85	0.84	0.81	0.85	0.84	0.83	0.81	0.83	0.84	0.85	0.83	0.83	0.81	0.83	0.82	0.85	0.80	0.81	0.81	0.83	0.82	0.79	0.80	0.82	0.82	0.84	0.83	0.83	0.84	0.84	0.83	0.82	0.84	0.84	0.83	0.81	0.83	0.86	0.86	0.87	0.83	0.83	0.82																																		
49	0.83	0.82	0.85	0.83	0.82	0.81	0.81	0.84	0.82	0.82	0.84	0.83	0.83	0.81	0.84	0.82	0.83	0.84	0.82	0.85	0.84	0.84	0.84	0.82	0.82	0.82	0.81	0.79	0.81	0.83	0.85	0.85	0.84	0.83	0.82	0.84	0.83	0.84	0.85	0.80	0.84	0.81	0.85	0.82	0.86	0.84	0.86	0.82																																		
50	0.81	0.83	0.85	0.81	0.84	0.82	0.80	0.82	0.81	0.82	0.81	0.80	0.84	0.82	0.80	0.79	0.80	0.83	0.81	0.82	0.81	0.80	0.80	0.81	0.81	0.79	0.79	0.80	0.81	0.82	0.82	0.81	0.80	0.80	0.82	0.84	0.85	0.84	0.84	0.79	0.86	0.86	0.84	0.84	0.82	0.85	0.83	0.85	0.81																																		
51	0.84	0.85	0.83	0.83	0.82	0.81	0.81	0.81	0.83	0.82	0.81	0.81	0.82	0.81	0.83	0.81	0.82	0.86	0.82	0.83	0.82	0.80	0.83	0.82	0.84	0.81	0.80	0.81	0.80	0.85	0.83	0.83	0.82	0.85	0.82	0.81	0.85	0.85	0.81	0.83	0.81	0.83	0.89	0.83	0.95	0.83	0.82	0.86	0.82	0.83																																		
52	0.82	0.84	0.85	0.84	0.85	0.86	0.81	0.84	0.83	0.83	0.81	0.83	0.84	0.82	0.83	0.82	0.83	0.84	0.82	0.82	0.80	0.80	0.83	0.80	0.84	0.79	0.78*	0.80	0.82	0.85	0.83	0.82	0.84	0.83	0.83	0.81	0.84	0.83	0.83	0.81	0.80	0.85	0.87	0.84	0.83	0.81	0.83	0.86	0.82	0.84	0.82																																		
53	0.83	0.82	0.84	0.83	0.83	0.82	0.80	0.83	0.82	0.83	0.81	0.82	0.84	0.82	0.83	0.82	0.81	0.84	0.83	0.84	0.81	0.84	0.82	0.83	0.83	0.80	0.81	0.80	0.80	0.84	0.86	0.86	0.85	0.84	0.82	0.84	0.87	0.84	0.86	0.80	0.83	0.82	0.84	0.85	0.84	0.84	0.87	0.85	0.90	0.84	0.84	0.83

4. Conclusions

PCA conducted on morphological characters resulted in a total diversity value of 64.67% and identified four main components. In addition, 772 SSR alleles with an average of 45 alleles per SSR locus were detected. The average heterozygosity was 0.83, and the average polymorphic information content (PIC) value was 0.96. All SSR markers showed a PIC value > 0.8, indicating their informativeness in analyzing genetic diversity of soybean. The phylogenetic analysis indicated a genetic similarity of 82% and grouped the accessions into two main clusters. The phylogenetic analysis showed that several accessions were grouped based on the growth type and origin. Two accessions that showed low genetic similarity of 78% (China cult-55 and Mi yang niu mao huang) can be selected as parents for plant breeding programs based on phylogenetic analysis and PCoA.

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