

Article

# Genetic Diversity Patterns and Discrimination of 172 Korean Soybean (*Glycine max* (L.) Merrill) Varieties Based on SSR Analysis

Tae-Young Hwang <sup>1</sup>, Byeong Sam Gwak <sup>2</sup>, Jwakyung Sung <sup>3,\*</sup>,<sup>†</sup> and Hong-Sig Kim <sup>3,\*</sup>,<sup>†</sup>

<sup>1</sup> International Technology Cooperation Center, Rural Development Administration, Jeonju, Jeollabuk-do 54875, Korea; soybeanhwang@korea.kr

<sup>2</sup> Gyeongsangbuk-do Silkworm & Insect Management Center, Sangju 37110, Korea; 95042040@daum.net

<sup>3</sup> Department of Crop Science, College of Agriculture, Life Science and Environmental Chemistry, Chungbuk National University, Cheongju, Chungcheongbuk-do 28644, Korea

\* Correspondence: jksung73@chungbuk.ac.kr (J.S.); hongsigk@chungbuk.ac.kr (H.-S.K.); Tel.: +82-43-261-2512 (J.S.); +82-43-261-2513 (H.-S.K.)

† These authors contributed equally to this article.

Received: 10 February 2020; Accepted: 11 March 2020; Published: 13 March 2020



**Abstract:** The soybean development goal in Korea has changed over time, but the pattern of genetic diversity in modern varieties has not yet been well characterized. In this study, 20 simple sequence repeat (SSR) markers are shown to generate a total of 344 alleles, where the number of alleles ranges from 7 to 29, with an average of 17.2 per locus, and the polymorphism informative content (PIC) values range from 0.6799 to 0.9318, with an average of 0.8675. Five different clusters are classified using the unweighted pair group mean arithmetic (UPGMA) method. The genetic distance between clusters I and V (0.3382) is the farthest, and that between clusters III and IV (0.0819) is the closest. The genetic distance between all pairings of groups, according to the time period of their release, is lowest (0.1909) between varieties developed in the 1990s and those from 2000 onward, and highest (0.5731) between varieties developed in the 1980s and those from 2000 onward. Model-based structure analysis revealed the presence of three sub-populations and 17 admixtures in the Korean soybean varieties. All 172 Korean soybean varieties were tested for discrimination using six SSR markers. The numbers of varieties that were clustered in each step are as follows: 7 (4.1%) in step 1 (Sat\_076), 73 (42.4%) in step 2 (Sat\_417), 69 (40.1%) in step 3 (Sat\_043), 13 (7.6%) in step 4 (Satt197), 8 (4.6%) in step 5 (Satt434), and 2 (1.2%) in step 6 (Satt179). These results, based on the analysis of genetic resources, can contribute to the creation of a core collection for soybean conservation and breeding, as well as to the development of future varieties with useful traits.

**Keywords:** soybean; genetic diversity; discrimination; population structure; simple sequence repeat (SSR); period of release

## 1. Introduction

Soybean (*Glycine max* (L.) Merrill) has historically been an important source of protein and oil for Korean people and is widely grown in East Asia, including in Northeast China [1]. The early cultivation of soybean in Korea is estimated to have begun around the fourth to fifth century BCE, and, since the era of the Three Kingdoms, it has become common in the Korean diet. In Korea, the goal of the development of soybean varieties was to increase yield up to the 1960s and 1970s. In the 1980s, mechanization-adaptive varieties were developed, in response to the declining labor force in rural areas. In the 1990s, soybean varieties were developed with the goal of breeding for diversification of use, multiplicity, and high quality, to allow for differentiation from imported varieties. In recent

years, the target when breeding soybean varieties has shifted to developing soybean for various uses; for example, as high-quality functional food or eco-friendly, safe, and insect- and disease-resistant varieties for stable production and to secure variety in genetic material for future needs [2,3]. The genetic diversity of crops or genetic resources used in breeding, as well as their inter-relationships, are crucial for increasing genetic variation, efficiently breeding, hybridizing elite breeding materials with close genetic distances [4], and increasing genetic resistance to insect pests or abiotic stresses [5].

Discrimination of variety is important for distinguishing between domestic and foreign agricultural products. Korea ratified the International Union for the Protection of New Varieties of Plants (UPOV) in 1991, as well as effectuating the Seed Industry Act in 1997 and the Nagoya Protocol in 2012, covering legislation on biodiversity conservation and utilization. For the rapid and accurate discrimination of varieties, techniques are required to protect breeder's rights. However, in any crop, old varieties are replaced by new varieties and, so, the discrimination of varieties is challenging. Discrimination of variety is complicated by agronomic characteristics, the natural environment, and social factors. Therefore, the discrimination of varieties is important for distinguishing between varieties, as well as to maintain the purity of the varieties. Bang et al. [6] reported that simple sequence repeat (SSR) markers can be used to test the distinction, uniformity, and stability of varieties for consistent identification and characterization of collected varieties, according to their origin and purity of seeds, allowing for the protection of varieties and the establishment of a seed management system. In recent years, the use of molecular markers in varietal development has increased, where it is applied for the evaluation of genetic diversity and variation.

Collecting various genetic resources and selecting excellent traits through botanical classification, external morphological characteristics, and genetic evaluation is important [7]. Many scientists have previously used morphological characteristics to analyze the genetic diversity and relationships between crop varieties and genetic resources; however, molecular markers are the most popular tool in genetic analysis at present. Molecular markers, including restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), randomly amplified polymorphic DNA (RAPD), SSR, and single nucleotide polymorphism (SNP) markers have been developed and are actively used in genetic diversity analysis, discrimination of varieties, and the detection and cloning of useful genes. As these molecular markers are not affected by the environment nor numerically limited, they can be effectively applied in genetic resource management and for the analysis of diversity in quantitative traits [8–11].

The identification of genetic structure using molecular markers, as well as morphological characteristics, is necessary for the efficient use of genetic resources. SSR markers are widely distributed across the genome, and their use as markers is based on differences in repetition of simple motifs; typically two or three nucleotide bases (e.g., AT<sub>23</sub>) [11,12]. SSR markers are two or three simple nucleotide sequences (microsatellites) which are widely distributed throughout the genome and their use as markers is based on differences in repetition. The value of polymorphism informative content (PIC) is considered high if a utility value is produced with many alleles in each locus, representing an ideal situation when used for identifying genetic diversity [13]. The aim of this study is to analyze the genetic diversity and inter-relationship, according to the release year of developed soybean varieties in Korea using SSR markers, in order for this information to be used to discriminate varieties and as basic data for future breeding activities.

## 2. Materials and Methods

### 2.1. Plant Material

A total of 172 varieties of soybean—which are used for sauce and paste (81 varieties), as bean sprouts (52 varieties), for cooking with rice (24 varieties), and as vegetables and early maturation (15 varieties)—have been developed in Korea from 1913 to 2013 (Table 1 and Table S1).

**Table 1.** List of 172 Korean soybean varieties, classified by release years and use type.

Release Period	Use Type				Total
	Soy Sauce and Tofu	Bean Sprout	Cooking with Rice	Vegetable and Early Maturity	
Before 1980	Jangdanbaekmok, Chungbukbaek, Iksan, Buseok, Haman, Keumkangdaelip, Geumgangsori, Yukwoo 3, Kwangdu, Shelby, Kwangkyo, Bongeui, Kanglim, Dongpuktae, Baegcheon, Jangyeob	Hill, Danyeob			18
1980s	Hwangkeum, Jangbeak, Namcheon, Deokyu, Milyang, Baegun, Saeal, Paldal, Pokwang, Dankyeong, Muhan, Jangkyong, Jangsu, Danweon	Pangsa, Eunha, Namhae			17
1990s	Malli, Shinpaldal, Samnam, Sinpaldal2, Taekwang, Duyou, Danbaek, Soyang, Jinpum, Geumgang, Dajang, Alchan, Jinpum2, Jangmi, Daewon, Ilmi, Sodam, Songhag, Daehwang	Bukwang, Hannam, Kwangan, Pureun, Sobaegnamul, Myeongjunamul, Iksannamul, Pungsannamul, Tawon, Somyeong, Paldo, Sowon, Doremi	Geomjeong1, Geomjeong2, Ilpumgeomjeong, Heugcheong, Jinyul, Galmi	Hwaecomput, Hwasongput, Geomjeongol, Saeol, Seonheuk	43
After 1999 (2000–2013)	Jangwon, Jinmi, Daepung, Daol, Hojang, Daemang, Sunyu, Daemang2, Hoban, Socheong, Nokwon, Jonam, Cheonga, Daewang, Gangil, Nampung, Manpoong, Daeha1, Daeha, Gyeongsang3, Wonyul, Soheug, Haessal, Cheonsang, Uram, Saedanbaek, Yeonpung, Jinyang, Wonhyun, Neulchan, Jang Yon, Gipung	Saebyeol, Soho, Sorog, Anpyeong, Dachae, Seonam, Dagi, Shingi, Sojin, Bosuk, Sokang, Nogchae, Aga1, Aga2, Wonhwang, Jangki, Youngyang, Soyong, Mansu, Pungwon, Pulmu-heukchae, Daeheug, Aga3, Aga4, Wonkwang, Hoseo, Shinhwa, Galchae, Sohwang, Pulmujigi, Wonheug, Aga8, Aga9, Aga10	Cheongja, Geomjeong3, Geumjeong4, Cheongdu1, Cheongja2, Cheongja3, Gyeongsang2, Ilpumgeomjeong2, Gyeongsang1, Josaengseori, Gaechuk1, Gaechuk2, Heugmi, Daeyang, Heugseong, Jinnong, Geomjeong5, Socheong2	Seonnog, Shillok, Danmi, Dajin, Geomjeongsaeol, Mirang, Danmi2, Sangwon, Hanol, Cheongyeob1	94
Total	81	52	24	15	172

## 2.2. DNA Extraction and SSR Analysis

Genomic DNA was extracted using the CTAB method [14]. The extracted DNA was used for PCR analysis by first checking the DNA concentration using a spectrophotometer (Nanodrop ND-1000, Thermo Scientific, Waltham, MA, USA) and adjusting the concentration to 20 ng/μL.

A total of 20 markers were used for the genetic diversity analysis (Table 2), selected from previous studies as having one primer with high polymorphism in each linkage group [9,15]. PCR was performed in a reaction mixture of 20 μL containing 20 ng of total genomic DNA, 0.4 μM of each primer, 10 mM dNTP mixture, 10 × PCR buffer, and 1 unit Taq polymerase (Bioneer, Daejeon Korea) using a MG96G (Longgene scientific, Hangzhou, China). The amplification protocol included initial denaturation for 5 min at 94 °C; 35 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 47–57 °C, and extension for 60 s at 72 °C; and a final extension step for 10 min at 72 °C. Amplification products were resolved using electrophoresis through 4% polyacrylamide sequencing gel at 1600 V for 100 to 120 min in 0.5 × Tris-borate-ethylenediaminetetraacetic acid (TBE, Skælskør, Denmark) buffer. The gel was stained with silver staining [16].

**Table 2.** Information of the 20 markers used for simple sequence repeat (SSR) analysis in this study.

Marker	Linkage Group	Chromosome No.	Repeat Motif	AT <sup>+</sup> (°C)	Sequence (5' to 3')
Sat_374	A1	05	(AT) <sub>23</sub>	57	F: GCGTTGAAACCGTTATAAACCAACTCA R: GCGCTTTATTGGCAATACTTTAACTCACAT
AW132402	A2	08	(AT) <sub>17</sub>	54	F: GCGCCTCCCTCCTCCTTTCTT R: GCGTTTCCCACATATTCTATCATTGT
Satt197	B1	11	(ATT) <sub>20</sub>	49	F: CACTGCTTTTTCCCCTCTCT R: AAGATACCCCCAACATTATTGTAA
Sat_342	B2	14	(AT) <sub>11</sub>	53	F: GCGACTCTGGGGAAAATTAGTTTAG R: GCGGAGTCGGGGAGCACTACTTGTC
Satt164	C1	04	(ATT) <sub>16</sub>	47	F: CACCAATGGCTAAAGGTACATAT R: AGGAGAAGAAAAATCACATAAAATATC
Sat_076	C2	06	(AT) <sub>40</sub>	47	F: GCGTAATTAACACCAATATATGACATG R: GCGGGGTAAAAAATTCAAAATGT
Satt179	D1a	01	(ATT) <sub>25</sub>	48	F: GGGATTAGGTTTATGGAAGTTTATTAT R: GGGTCATTAAAACGATCAGTAAGA
Satt216	D1b	02	(ATT) <sub>19</sub>	47	F: TACCCTTAATCACCGGACAA R: AGGGAACAAACACATTTAATCATCA
Sat_022	D2	17	(AT) <sub>27</sub>	47	F: GCGGCCTTTTCTGACTGTAA R: GCGCAGTGACTAAAACCTTACTAT
Satt185	E	15	(ATT) <sub>29</sub>	49	F: GCGCATATGAATAGGTAAGTTGCACTAA R: GCGTTTTCTACAATAATATTTTCAT
Sat_417	F	13	(AT) <sub>16</sub>	54	F: GCGAATATGGCGTTGAAAATAGTGAT R: GCGACCCAGATTCTGTGCTAAGA

Table 2. Cont.

Marker	Linkage Group	Chromosome No.	Repeat Motif	AT <sup>†</sup> (°C)	Sequence (5' to 3')
Sat_210	G	18	(AT) <sub>35</sub>	52	F: GCGCCAGCAACAAAGTTCCTGACAAA R: GCGCATGCAAATGAAATAATAA
Satt434	H	12	(ATT) <sub>32</sub>	48	F: GCGTTCGATATACTATATAATCCTAAT R: GCGGGGTAGTCTTTTTATTTAACTTAA
Sat_105	I	20	(AT) <sub>30</sub>	47	F: TTCCATACAAGATATCAAGTGAATTG R: GCTCCCCTACATTGGTAGTAAA
Sat_151	J	16	(AT) <sub>13</sub>	50	F: GCTGCATCAGATCACCCATCCTTC R: CATGCCATGTTGTATGTATGT
Sat_043	K	09	(AT) <sub>23</sub>	53	F: GCGGTCCGTCAATGAATATTAATTAATAA R: GCGAAAGCGGCAGAGAGAGAAAGGT
Sat_245	L	19	(AT) <sub>27</sub>	55	F: GCGAGCCTACTTTTACTAGAACGTCAACAAG R: GCGAAAAATTCAACTCCCCTTTAATAGATTC
Sat_391	M	07	(AT) <sub>37</sub>	52	F: GCGTAGGCATCGGTCAATATTTT R: GCGTTAGCGAGTGGATCAAGATCA
Satt339	N	03	(ATT) <sub>26</sub>	49	F: TAATATGCTTTAAGTGGTGTGGTTATG R: GTTAAGCAGTTCCTCTCATCACG
Sat_190	O	10	(AT) <sub>9</sub>	51	F: GCGTGGAATAATTTTAAAGATTAGGA R: GCGTGTGTACATTAGTCTACTTGAGAA

<sup>†</sup> annealing temperature.

### 2.3. Selection of SSR Markers for Discrimination of the Varieties

The 172 varieties of Korean soybean were discriminated in six steps using six markers, including Sat\_076, Sat\_417, Sat\_043, Satt197, and Satt434 with high polymorphism, and one marker, Satt179, with the lowest polymorphism. The discrimination of soybean varieties was determined by combining the markers with the highest polymorphism in the first step and the marker with the next highest polymorphism in each step. Four markers (Sat\_076, Sat\_417, Sat\_043, and Satt197), which were highly diverse among the 20 markers [9], were initially selected. Additional markers (Satt434 and Satt179) were selected to efficiently identify the unknown varieties.

### 2.4. Data Analysis

The number of alleles, major allele frequency, genetic diversity, and PIC values were analyzed using PowerMarker 3.25 software [17]. Genetic distances were obtained using PowerMarker, according to the method of Nei [18], and the phylogenetic tree was prepared using the unweighted pair group mean arithmetic (UPGMA) method to compare group classification.

The software program Structures 2.3.3 [19,20] was utilized to detect possible sub-populations ( $K = 1$  to  $K = 10$ ) with a model allowing for admixture and correlated allele frequencies using a burn-in of 100,000 and run length of 100,000, followed by 5 iterations. The optimal number of populations corresponds to the highest peak in the  $\Delta K$  graph [21], and the Korean soybean varieties with membership probabilities (more than 90%) were assigned to each sub-population.

## 3. Results

### 3.1. Genetic Diversity and Polymorphism of SSR Loci

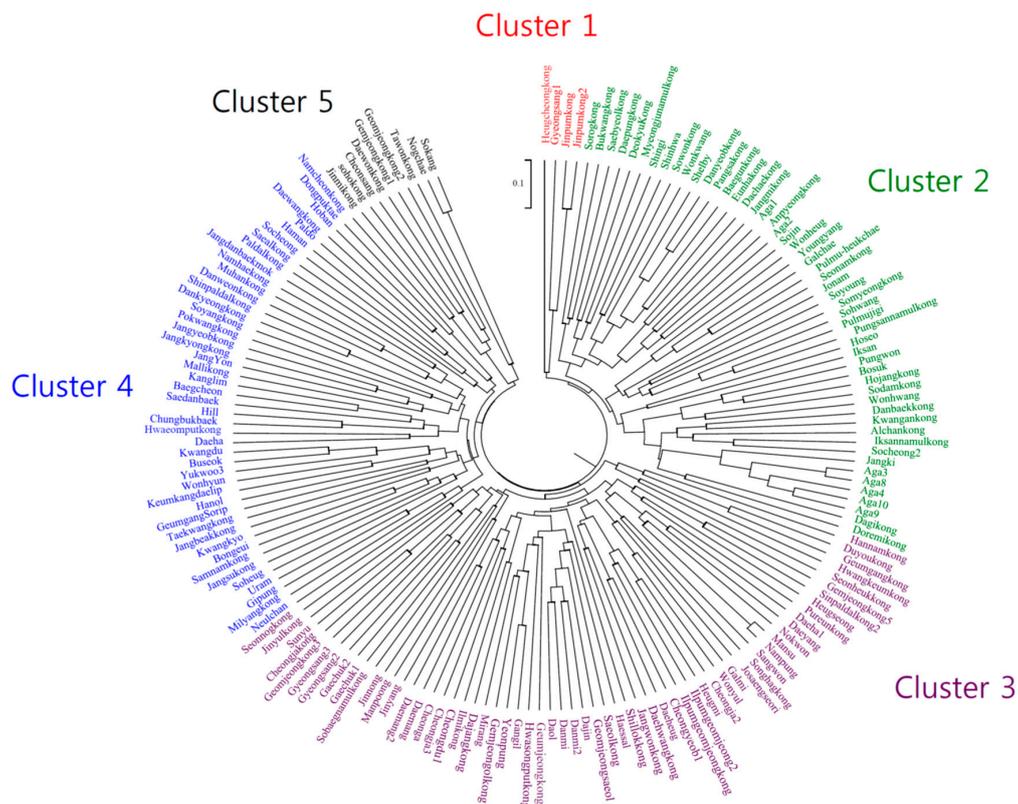
Table 3 shows the results of genetic diversity analysis using the 20 SSR markers for the 172 soybean varieties developed in Korea. A total of 344 alleles were detected. The number of alleles ranged from 7 (Satt164 and Satt179) to 29 (Sat\_076) per locus, with an average of 17.2. The size of alleles ranged from 107 to 361 bp, with Sat\_417 and Sat\_190 at the widest range of 139–229 bp and 125–215 bp, respectively; whereas Sat\_164 had the narrowest range of 222–246 bp (Table S2). PIC values ranged from 0.6799 to 0.9318, with Sat\_076 having the highest (0.9318) and Satt179 the lowest (0.6799). The average PIC value was 0.8675.

The total varieties per cluster were as follows: 4 (2.3%) in cluster I, 52 (30.2%) in cluster II, 61 (35.5%) in cluster III, 46 (26.7%) in cluster IV, and 9 (5.2%) in cluster V. More than 92% of the total varieties were found in three clusters (II, III, and IV; Figure 1, Table S1 and Table 4). Calculation of genetic distance for pairwise combinations of all soybean varieties supported this conclusion (Table 5). Although the results were obtained with only a limited number of genotypes, clusters III and IV showed the lowest genetic distance (Table 5).

**Table 3.** Linkage group analysis of 20 simple sequence repeat (SSR) markers used for genotyping in 172 Korean soybean varieties.

Marker	Size (bp)	N <sub>A</sub> <sup>†</sup>	N <sub>AF</sub> <sup>‡</sup>	PIC <sup>§</sup>
Sat_374	273–343	21	0.1919	0.9060
AW132402	137–183	15	0.1977	0.8630
Satt197	107–182	16	0.1395	0.9126
Sat_342	176–246	19	0.2035	0.9113
Satt164	222–246	7	0.3256	0.7529
Sat_076	122–208	29	0.1337	0.9318
Satt179	130–184	7	0.4244	0.6799
Satt216	164–248	15	0.3430	0.7850
Sat_022	203–255	18	0.1919	0.9114
Satt185	210–270	16	0.1919	0.8819
Sat_417	139–229	22	0.1105	0.9267
Sat_210	217–287	18	0.2151	0.8741
Satt434	301–361	18	0.2326	0.8875
Sat_105	228–274	18	0.1628	0.9067
Sat_151	210–272	17	0.2849	0.8241
Sat_043	242–294	18	0.1453	0.9167
Sat_245	119–207	19	0.1395	0.9107
Sat_391	214–282	16	0.1628	0.8903
Satt339	203–245	14	0.2849	0.8041
Sat_190	125–215	21	0.2616	0.8737
Total		344		
Mean		17.2	0.2172	0.8675

<sup>†</sup> number of alleles; <sup>‡</sup> major allele frequency; <sup>§</sup> polymorphic information content.



**Figure 1.** Unweighted pair group mean arithmetic (UPGMA) phylogenetic tree using 20 SSR markers in 172 Korean soybean varieties.

**Table 4.** List of varieties in each cluster classified by release years using SSR markers in 172 Korean soybean varieties.

Cluster	Release Period				Total
	Before 1980	1980s	1990s	2000 or Later	
I			Jinpum, Jinpum2, Heugcheong	Gyeongsang1	4 (2.3%)
II	Iksan, Shelby, Danyeob,	Deokyu, Baegun, Pangsa, Eunha,	Bukwang, Danbaek, Kwangan, Myeongjunamul, Iksannamul, Alchan, Pungsannamul, Jangmi, Somyeong, Sodam, Sowon, Doremi,	Saebyeol, Sorog, Anpyeong, Daepung, Dachae, Seonam, Dagi, Hojang, Shingi, Sojin, Bosuk, Aga1, Aga2, Wonhwang, Jangki, Youngyang, Soyyoung, Pungwon, Jonam, Pulmu-heukchae, Aga3, Aga4, Wonkwang, Hoseo, Shinhwa, Galchae, Sohwan, Pulmujigi, Wonheug, Socheong2, Aga8, Aga9, Aga10	52 (30.2%)
III		Hwangkeum	Sinpaldal2, Duyou, Hwasongput, Hannam, Pureun, Geumgang, Sobaegnamul, Dajang, Gemjeongol, Ilpumgeomjeong, Ilmikong, Saeol, Seonheuk, Songhag, Jinyul, Daehwang, Galmi	Jangwon, Cheongja, Seonnog, Shillok, Geomjeong3, Geumjeong4, Daol, Danmi, Cheongdu1, Daemang, Dajin, Cheongja2, Cheongja3, Sunyu, Geomjeongsaeol, Gyeongsang2, Daemang2, Mirang, Danmi2, Ilpumgeomjeong2, Josaengseori, Gaechuk1, Gaechuk2, Heugmi, Nokwon, Mansu, Cheonga, Gangil, Nampung, Sangwon, Daeheug, Daeyang, Manpoong, Heugseong, Daeha1, Gyeongsang3, Jinnong, Gemjeong5, Cheongyeob1, Wonyul, Haessal, Yeonpung, Jinyang	61 (35.5%)
IV	Jangdanbaekmok, Chungbukbaek, Buseok, Haman, Keumkangdaelip, GeumgangSorip, Yukwoo3, Kwangdu, Hill, Kwangkyo, Bongeu, Kanglim, Dongpuktae, Baegcheon, Jangyeob	Jangbeak, Namcheon, Milyang, Saeal, Paldal, Pokwang, Dankyeong, Muhan, Namhae, Jangkyong, Jangsu, Danweon,	Malli, Shinpaldal, Samnam, Taekwang, Hwaecomput, Soyang, Paldo,	Hoban, Socheong, Daewang, Daeha, Hanol, Soheug, Uram, Saedanbaek, Wonhyun, Neulchan, JangYon, Gipung	46 (26.7%)
V			Gemjeong1, Geomjeong2, Tawon, Daewon	Soho, Jinmi, Sokang, Nogchae, Cheonsang	9 (5.2%)
Total	18	17	43	94	172 (100%)

Note: Value in brackets correspond to the percentage distribution of varieties in each cluster classified according to release year.

**Table 5.** Genetic distance between all pairings of clusters based on SSR analysis.

Cluster	I	II	III	IV	V
I	-				
II	0.2412	-			
III	0.2451	0.0873	-		
IV	0.2482	0.0915	0.0819	-	
V	0.3382	0.1659	0.1459	0.1473	-

### 3.2. Polymorphism of SSR Loci by Time Period of Release Years and Population Structure

The number of alleles and PIC values obtained as a result of our SSR analysis of the 172 Korean soybean varieties of each group, as classified by release year, are shown in Table 6. A total of 185 alleles in Korean soybean varieties were developed before 1980, where the average number of alleles was 9.3 and the average PIC value was 0.8188. In the 1980s, the total number of alleles was 167, the average number of alleles was 8.4, and the average PIC value was 0.7913. A total of 251 alleles in

Korean soybean varieties were developed in the 1990s, where the average number of alleles was 12.6 and the average PIC value was 0.8416. Since 2000, the allele total was 315, the average number of alleles was 15.8, and PIC value was 0.8631. The soybean varieties developed after the 1990s and 2000s had a higher number of alleles and higher PIC values than those developed before 1980. Due to the sample size difference, the varieties developed after the 1990s and 2000s had more alleles than the varieties developed before 1980.

**Table 6.** Information of 20 markers used for SSR analysis by release period.

Marker	Release Period							
	Before 1980		1980s		1990s		2000 or later	
	N <sub>A</sub> . <sup>†</sup>	PIC <sup>‡</sup>	N <sub>A</sub> .	PIC	N <sub>A</sub> .	PIC	N <sub>A</sub> .	PIC
Sat_374	9	0.8143	9	0.8198	17	0.9006	20	0.8999
AW132402	8	0.7941	7	0.7928	10	0.8383	13	0.8604
Satt197	10	0.8431	7	0.7713	13	0.8890	15	0.9121
Sat_342	10	0.8431	10	0.8288	15	0.8724	19	0.9028
Satt164	4	0.6658	6	0.7817	7	0.7810	7	0.7188
Sat_076	13	0.9001	13	0.8846	19	0.9165	24	0.9285
Satt179	5	0.6987	6	0.5299	5	0.5851	6	0.7093
Satt216	8	0.6886	6	0.5769	10	0.8074	11	0.7779
Sat_022	12	0.8795	9	0.8032	15	0.8927	18	0.9116
Satt185	12	0.8929	9	0.8427	11	0.8395	15	0.8727
Sat_417	8	0.8197	11	0.8752	17	0.9127	20	0.9252
Sat_210	10	0.8645	9	0.8427	12	0.7753	16	0.8778
Satt434	9	0.8024	9	0.8343	13	0.8682	17	0.8904
Sat_105	9	0.8422	8	0.8339	12	0.8724	17	0.9074
Sat_151	7	0.7900	7	0.7157	10	0.7695	16	0.8350
Sat_043	10	0.8640	11	0.8830	15	0.9003	17	0.8982
Sat_245	12	0.8724	6	0.7907	14	0.8989	18	0.9052
Sat_391	11	0.8649	9	0.8266	12	0.8470	15	0.8889
Satt339	8	0.7704	5	0.7557	11	0.8034	12	0.8001
Sat_190	10	0.8645	10	0.8372	13	0.8612	19	0.8399
Total	185		167		251		315	
Mean	9.25	0.8188	8.35	0.7913	12.55	0.8416	15.75	0.8631

<sup>†</sup> number of alleles; <sup>‡</sup> polymorphic information content.

The varieties in each cluster, as classified by release period, are shown in Table 4. We found that 18 varieties developed before 1980 were distributed in clusters II and IV, with the most varieties (15) occurring in cluster IV. The 17 varieties developed in the 1980s were in clusters II, III, and IV, with most of these (12) in cluster IV. The 43 varieties developed in the 1990s were distributed throughout all clusters, with 17 varieties found in cluster IV. The varieties developed since the 2000s were distributed in all clusters, but mostly in clusters II and III (80%).

The genetic distances between all pairings of groups by release period are shown in Table 7. The genetic distance between varieties developed after 2000 and those developed in the 1980s was the furthest (0.5731), while the genetic distance between varieties developed after 2000 and those developed in the 1990s was the closest (0.1909; Table 7).

**Table 7.** Genetic distance between all pairings of group by release period based on SSR analysis.

Release Period	Before 1980	1980s	1990s	2000 or Later
Before 1980	-			
1980s	0.3382	-		
1990s	0.4906	0.4444	-	
2000 or later	0.5587	0.5731	0.1909	-

The population structure of the 172 Korean soybean varieties was inferred using the Structure V2.3.3 software, based on 20 SSR markers. For each K value ( $K = 1-10$ ), we determined the optimal value of K by calculating  $\Delta K$ . As we used Korean soybean varieties in this study, we surveyed the highest  $\Delta K$  value over “ $K = 3$ ”. The three sub-populations, referred to as Pop. 1–3, were observed with the highest  $\Delta K$  value (Figure 2) and 17 varieties were admixture type, with a membership probability < 90% (Figure 3). Most soybean varieties were distinguished according to release period. The result of population structure analysis is shown in Table 8. We found that only 18 varieties developed before or in the 1980s were in Pop. 3, except Iksan and Hwangkeumkong. The varieties developed since the 2000s were distributed in all clusters, but mostly in Pops. 1 and 2 (79%).

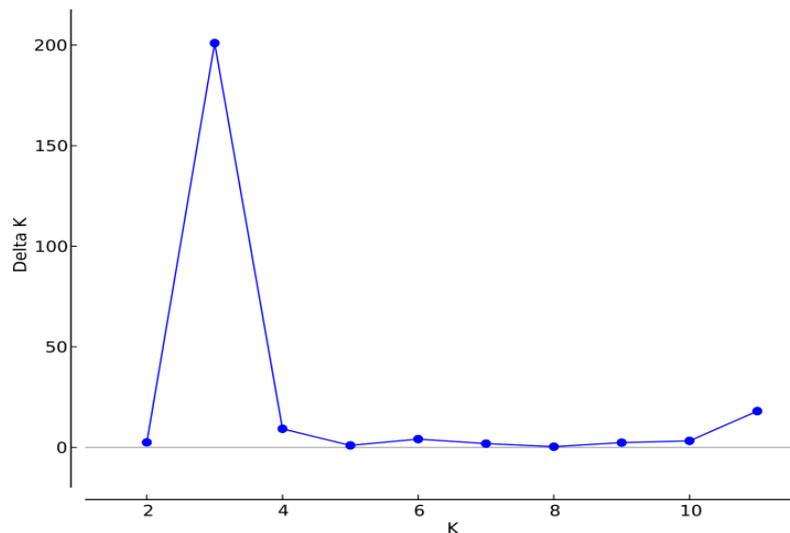


Figure 2. Values of  $\Delta K$ , with its modal value detecting a true K of three groups ( $K = 3$ ).

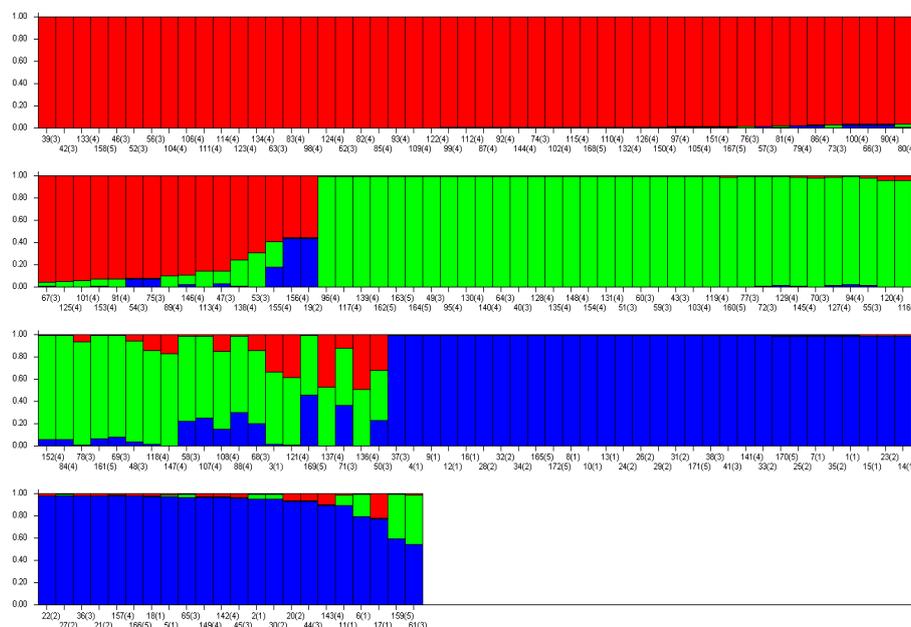


Figure 3. Model-based membership of 172 Korean soybean varieties using STRUCTURE. Colors represent model-based sub-populations as follows: red, Pop. 1; lime, Pop. 2; and blue, Pop. 3.

**Table 8.** Each step of discrimination of 172 Korean soybean varieties using six SSR markers.

Step	Marker	Identified Varieties	No. of Identified Varieties	Percentage of Identified Varieties
Step 1	Sat_076	Gaechuk2, Geumjeong4, Heugcheong, Jinnong, Manpoong, Seonam, Sobaegnamul	7	4.1%
Step 2	Step 1 + Sat_417	Bongeu, Galmi, Geumgangsori, Jangkyong, Somyeong, Heugseong, Jangyon, Jangsu, Pureun, Baegcheon, Daemang2, Dankyeong, Deokyu, Haessal, Taekwang, Ilpumgeomjung, Jangdanbaekmok, Wonkwang, Saedanbaek, Tawon, Wonheug, Dajang, Geomjeongol, Cheongja2, Cheongyeob1, Daehwang, Galchae, Paldo, Pokwang, Shinpaldal, Jinyang, Milyang, Wonhwang, Aga2, Samnam, Sohwan, Sorog, Pulmujigi, Shingi, Haman, Hwaeomput, Heugmi, Jangyeob, Malli, Jangwon, Dongpuktae, Jonam, Saeal, Soyoung, Bukwang, Daepung, Pungsannamul, Saebyeol, Jangmi, Kwangan, Muhan, Sangwon, Shinhwa, Youngyang, Hoban, Gyeongsang1, Jangbeak, Shelby, Wonhyun, Josaengseori, Pangsa, Doremi, Hanol, Danyeob, Hojang, Hoseo, Namcheon, Soho	73	42.4%
Step 3	Step 2 + Sat_043	Duyou, Geumgang, Cheongja, Geomjeong1, Daewon, Shillok, Ilpumgeomjeong2, Sunyu, Cheonga, Cheongja3, Sowon, Sodam, Alchan, Dachae, Iksannamul, Socheong2, Dagi, Songhag, Danbaek, Anpyeong, Eunha, Baegun, Gyeongsang2, Gyeongsang3, Gaechuk1, Aga4, Aga9, Jangki, Paldal, Danmi2, Geomjeongsaeol, Hill, Chungbukbaek, Danweon, Jinpum, Jinpum2, Cheongdu1, Daemang, Daeyang, Geomjeong3, Buseok, Kwangdu, Nokwon, Seonnog, Daol, Jinyul, Gangil, Dajin, Pulmu-heukchae, Saeol, Geomjeong2, Mirang, Daeheug, Myeongjunamul, Danmi, Hannam, Soyang, Ilmi, Yukwoo 3, Kanglim, Kwangkyo, Jinmi, IksanCheonsang, Hwangkeum, Keumkangdaelip, Neulchan, Soheug, Wonyul	69	40.1%
Step 4	Step 3 + Satt197	Gipung, Uram, Daewang, Socheong, Hwasongput, Yeonpung, Aga10, Sojin, Aga1, Namhae, Seonheuk, Geomjeong5, Sinpaldal2	13	9.6%
Step 5	Step 4 + Satt434	Nogchae, Sokang, Aga3, Aga8, Pungwon, Bosuk, Daeha, Daeha1	8	4.6%
Step 6	Step 5 + Satt179	Mansu, Nampung	2	1.2%
	Total		172	100%

### 3.3. Discrimination of Soybean Varieties

In the first step, seven (4.1%) varieties were discriminated according to the results when using Sat\_076, which had the highest allele and PIC values, and were not discriminated from 165 varieties. In step 2, 73 (42.4%) varieties were discriminated among the 165 varieties by including Sat\_417. In step 3, 69 (40.1%) varieties were discriminated among 92 varieties by including Sat\_043. In step 4, 13 (7.6%) of 23 varieties were discriminated when including Satt197. In step 5, 8 (4.6%) of 10 varieties were discriminated when including Satt434. In the final step, two varieties (1.2%), Mansu and Nampung, were discriminated by including Sat\_179, allowing for discrimination of all 172 varieties. Of the 20 markers, Mansu and Nampung could only be discriminated when using Sat\_179 (Table 8, Figure S2).

## 4. Discussion

Many genetic diversity analyses using DNA markers have been reported for the development of new varieties, discrimination of varieties, and to search for useful genes. Song et al. [22] reported the use of 72 markers to analyze 185 accessions of genetic resources collected from Korea, China, Japan, India, Myanmar, Philippines, and the United States, detecting 3–31 alleles per locus and an average of 10.9 alleles, which were used to classify the data into three groups, following which detailed groups were formed according to geographical origins. Kuroda et al. [23] used the genetic resources of 1318

native varieties and wild-type plants from China, Japan, Korea, and Russia to analyze the genetic diversity and develop a core collection. A high number of rare alleles were found in the wild types of Korea, suggesting that the ratio of soybean core collection to selection was high. Korea has its own wild soybean types, but other types of resources have also been introduced from both China and Japan, resulting in accessions with high genetic diversity. Wang et al. [24] analyzed 23 developed soybean and native varieties and 17 wild types using 40 SSR markers, and detected that the high genetic diversity of wild types was due to the loss of many alleles in the evolutionary process.

The genetic distance between all pairings of groups by release period shows that those in the 1990s and 2000 onward were the closest (0.1909), and 2000 onward and before or during the 1980s were the farthest (0.5587 and 0.5731, respectively; Table 7). The PIC value, in terms of release periods among Korean soybean varieties, was the highest in those developed from 2000 onward (Table 6). The reason for this finding is that, since the mid-1980s, various varieties have been developed with the aim of diversification of use and high quality. Many varieties have been developed for comparison to other groups by release period. We also found that the most genetic diversity among varieties can be observed in those developed since the 2000s (Figure 1 and Figure S1, Table 4). This trend is expected to continue in the future.

Many results of genetic diversity analyses and discrimination of varietal studies using DNA markers have been reported. Kim et al. [25] discriminated 82 of the 91 developed soybean varieties from 1913 to 2002 using five SSR markers—Sat\_043, Sat\_022, Sat\_036, Sat\_088, and Satt045—and reported that unidentified varieties could not be distinguished using their morphological characteristics. Hwang et al. [26] reported that 18 sequence-tagged sites—cleaved amplification polymorphic sequence (STS-CAPS) markers (51 combinations)—were developed from base sequence information in prepared genomic DNA libraries, and 106 Korean soybean varieties were discriminated in 14 steps. Gao et al. [27] discriminated 83 soybean varieties using nine markers with high polymorphism and high diversity within the population. An allele of each marker was coded to form a nine-digit identification (ID) for the discrimination of soybean varieties.

In Korea, the first developed soybean variety was Jangdanbaekmok produced by separating landrace through pure line isolation in 1913, and the first hybrid variety was developed in 1969 characterized with high yielding ability and resistant to mosaic virus [28]. Until 2014, a total of 178 soybean varieties have been developed and registered at the two national institutes, the RDA-Genebank Information Center and the Korea Seed & Variety Service [28,29]. Lee et al. [29] analysis that a total of four pedigrees involving 168 varieties (94.4% out of 178 varieties), which form the broadest network of pedigrees. We analyzed genetic diversity using 172 Korean soybean varieties developed until 2013. Of these, 162 varieties include in four pedigrees, the results of genetic diversity and pedigrees provide information for selection of parental lines and design of crossing strategies.

The full-length sequence of the American soybean variety Willams 82 has been analyzed and published for the first time [30] and, using the sequence information, a large amount of information regarding candidate polymorphic SSR markers has also been published [31]. In Korea, Kim et al. [32] analyzed the full-length sequences of six Korean soybean varieties to distinguish genomes from dense variation blocks (dVBs) with SNP accumulation and sparse variation blocks with little SNPs. As dVBs have distinctive characteristics according to variety, various differences were indeed found to exist among the varieties. Therefore, 202 dVB-specific insertion/deletion (indel) markers have been developed [33]. A barcode system was established, which clearly distinguishes 147 Korean soybean varieties and is regularly updated whenever new varieties are developed [34,35]. The development of DNA markers has been simplified due to the active progression of genome research, but the development of DNA markers through full-length genome analysis in a small-scale laboratory is still difficult. As the marker analysis was not performed directly, polymorphism information is not known in full-length genome analysis. In this study, we analyzed 172 Korean soybean varieties developed from 1913 to 2013 using SSR markers that have been previously confirmed to be highly polymorphic. The analysis of genetic resources, including newly developed soybean varieties after 2014, in addition

to the data accumulated in this study, will contribute to the development of future varieties with useful traits, such as drought- and disease-resistance, including the determination of species and the creation of a core collection for soybean conservation and breeding.

## 5. Conclusions

In this study, the genetic diversity of 172 Korean soybean varieties was analyzed and discriminated using SSR Markers. Five different clusters were classified using the UPGMA method, among which the genetic distance between clusters I and V was the farthest, and that between clusters III and IV was the closest. The genetic diversity distance by release period between all pairings of groups was the lowest between varieties developed after 2000 and the 1990s, and the highest between varieties developed after 2000 and the 1980s. The results demonstrate that the greatest genetic diversity among varieties was found in those that had been developed after 2000. The 172 varieties of Korean soybean were discriminated in six steps using six markers (Sat\_076, Sat\_417, Sat\_043, Satt197, Satt434, and Satt179). The analysis of genetic resources, including newly developed varieties, in addition to the data accumulated in this study, will contribute to the creation of a core collection for soybean conservation and breeding, as well as to the development of future varieties with useful traits.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2077-0472/10/3/77/s1>, Table S1: Number of soybean varieties classified by released year and breeding organization for this experiment, Table S2: Information of band size (bp) based on 20 markers for SSR analysis in 172 Korean soybean varieties, Figure S1: Principal coordinate analysis (PCoA) plot of 172 Korean soybean varieties using 20 SSR markers, Figure S2: Discrimination of varieties at each step by UPGMA dendrogram using six markers in 172 Korean soybean varieties.

**Author Contributions:** Conceptualization, J.S. and H.-S.K.; Methodology, T.-Y.H. and B.S.G.; Formal analysis, T.-Y.H. and B.S.G.; Investigation, T.-Y.H., B.S.G., and J.S.; Resources, H.-S.K.; Data curation, T.-Y.H.; Writing—original draft preparation, T.-Y.H. and B.S.G.; Writing—review and editing, J.S. and H.-S.K. Visualization, T.-Y.H.; Supervision, J.S.; Project administration, H.-S.K.; Funding acquisition, H.-S.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** This work was financially supported by the Research Year of Chungbuk National University in 2018.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Lee, G.A.; Choi, Y.M.; Chung, J.W.; Lee, M.C.; Ma, K.H.; Lee, S.; Cho, J.W.; Lee, J.R. Genetic Diversity and Population Structure of Korean Soybean Collection Using 75 Microsatellite Markers. *Korean J. Crop Sci.* **2014**, *59*, 492–497. [[CrossRef](#)]
2. Choi, Y.M.; Lee, M.C.; Ro, N.Y.; Lee, S.G.; Gwag, J.G.; Yoon, M.S. Morphological Characteristics and SSR Profiling of Soybean Landraces of Korea. *Korean J. Breed. Sci.* **2014**, *46*, 353–363. [[CrossRef](#)]
3. Qiu, L.J.; Xing, L.L.; Guo, Y.; Wang, J.; Jackson, S.A.; Chang, R.Z. A Platform for Soybean Molecular Breeding: The Utilization of Core Collections for Food Security. *Plant Mol. Biol.* **2013**, *83*, 41–50. [[CrossRef](#)] [[PubMed](#)]
4. Tantasawat, P.; Trongchuen, J.; Prajongjai, T.; Jenweerawat, S.; Chaowiset, W. SSR Analysis of Soybean (*Glycine max* (L.) Merr.) Genetic Relationship and Variety Identification in Thailand. *Aust. J. Crop Sci.* **2011**, *5*, 283–290.
5. Li, Z.; Nelson, R.L. Genetic Diversity among Soybean Accessions from Three Countries Measured by RAPDs. *Crop Sci.* **2001**, *41*, 1337–1347. [[CrossRef](#)]
6. Bang, K.H.; Jo, I.H.; Chung, J.W.; Kim, Y.C.; Lee, J.W.; Seo, A.Y.; Park, J.H.; Kim, O.T.; Hyun, D.Y.; Kim, D.H.; et al. Analysis of Genetic Polymorphism of Korean Ginseng Cultivars and Foreign Accessions Using SSR Markers. *Korean J. Med. Crop Sci.* **2011**, *19*, 347–353. [[CrossRef](#)]
7. Zhou, Z.; Jiang, Y.; Wang, Z.; Gou, Z.; Lyu, J.; Li, W.; Yu, Y.; Fang, C. Resequencing 302 Wild and Cultivated Accessions Identifies Genes Related to Domestication and Improvement in Soybean. *Nat. Biotechnol.* **2015**, *33*, 408–414. [[CrossRef](#)]

8. Jang, S.J.; Park, S.J.; Park, K.H.; Song, H.L.; Cho, Y.G.; Jong, S.K.; Kang, J.H.; Kim, H.S. Genetic Diversity and Identification of Korean Soybean Cultivars Including Certified Cultivars Based on SSR Markers. *Korean J. Crop Sci.* **2009**, *54*, 231–240.
9. Hwang, T.Y.; Nakamoto, Y.; Kono, I.; Enoki, H.; Funatsuki, H.; Kitamura, K.; Ishimoto, M. Genetic Diversity of Cultivated and Wild Soybeans Including Japanese Elite Cultivars as Revealed by Length Polymorphism of SSR Markers. *Breed. Sci.* **2008**, *58*, 315–323. [[CrossRef](#)]
10. Jong, S.K.; Kim, S.H.; Woo, S.H.; Kim, H.S. Comparison of Pedigree-and DNA-Based Genetic Similarity among Korean Soybean Cultivars. *Korean J. Breed. Sci.* **2006**, *38*, 19–25.
11. Wang, L.; Guan, R.; Liu, Z.; Chang, R.; Qiu, L. Genetic Diversity of Chinese Cultivated Soybean Revealed by SSR Markers. *Crop Sci.* **2006**, *46*, 1032–1038. [[CrossRef](#)]
12. Ghosh, J.P.; Ghosh, D.; Choudhury, P.R. An Assessment of Genetic Relatedness Between Soybean [*Glycine max* (L.)/Merrill] Cultivars Using SSR Markers. *Am. J. Plant Sci.* **2014**, *5*, 3089–3096. [[CrossRef](#)]
13. Weising, K.; Winter, P.; Hüttel, B.; Kahl, G. Microsatellites Marker for Molecular Breeding. *J. Crop Prod.* **1998**, *1*, 113–143. [[CrossRef](#)]
14. Keim, P.; Olson, T.C.; Shoemaker, R.C. A Rapid Protocol for Isolating Soybean DNA. *Genet. Newsl.* **1988**, *15*, 150–152.
15. Jang, S.J.; Park, S.J.; Piao, X.M.; Song, H.L.; Hwang, T.Y.; Cho, Y.G.; Liu, X.H.; Woo, S.H.; Kang, J.H.; Kim, H.S. Genetic Diversity and Relationships of Korean, Japanese, and Chinese Jilin Provincial Wild Soybeans (*Glycine soja* Sieb. and Zucc.) Based on SSR Markers. *Korean J. Breed. Sci.* **2010**, *42*, 87–99.
16. Panaud, O.; Chen, X.; McCouch, S.R. Development of Microsatellite Markers and Characterization of Simple Sequence Length Polymorphism (SSLP) in Rice (*Oryza sativa* L.). *Mol. Gen. Genet.* **1996**, *252*, 597–607. [[CrossRef](#)]
17. Liu, K.; Muse, S.V. PowerMarker: An Integrated Analysis Environment for Genetic Marker Analysis. *Bioinformatics* **2005**, *21*, 2128–2129. [[CrossRef](#)]
18. Nei, M. Analysis of Gene Diversity in Subdivided Populations. *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 3321–3323. [[CrossRef](#)]
19. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **2000**, *155*, 945–959.
20. Falush, D.; Stephens, M.; Pritchard, K. Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics* **2003**, *164*, 167–187.
21. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the Number of Clusters of Individuals Using the Software STRUCTURE: A Simulation Study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [[CrossRef](#)] [[PubMed](#)]
22. Song, J.Y.; Piao, X.M.; Choi, Y.M.; Lee, G.A.; Chung, J.W.; Lee, J.R.; Jung, Y.J.; Park, H.J.; Lee, M.C. Evaluation of Genetic Diversity and Comparison of Biochemical Traits of Soybean (*Glycine max* L.) Germplasm Collections. *Plant Breed. Biotechnol.* **2013**, *1*, 374–384. [[CrossRef](#)]
23. Kuroda, Y.; Tomooka, N.; Kaga, A.; Wanigadeva, S.M.S.W.; Vaughan, D.A. Genetic Diversity of Wild Soybean (*Glycine soja* Sieb. et Zucc.) and Japanese Cultivated Soybeans [*G. max* (L.) Merr.] Based on Microsatellite (SSR) Analysis and the Selection of a Core Collection. *Gene. Resour. Crop Evol.* **2009**, *56*, 1045–1055. [[CrossRef](#)]
24. Wang, M.; Li, R.Z.; Yang, W.M.; Du, W.J. Assessing the Genetic Diversity of Cultivars and Wild Soybeans Using SSR Markers. *Afr. J. Biotechnol.* **2010**, *9*, 4857–4866.
25. Kim, S.H.; Chung, J.W.; Moon, J.K.; Woo, S.H.; Cho, Y.G.; Jong, S.K.; Kim, H.S. Discrimination of Korean Soybean Cultivars by SSR Markers. *Korean J. Crop Sci.* **2006**, *51*, 1–11.
26. Hwang, T.Y.; Seo, M.J.; Lee, S.K.; Park, H.M.; Jeong, K.H.; Lee, Y.Y.; Kim, S.L.; Yun, H.T.; Lee, J.E.; Kim, D.W.; et al. Discrimination of 110 Korean Soybean Cultivars by Sequence Tagged Sites (STS)-CAPS Markers. *Korean J. Breed. Sci.* **2012**, *44*, 258–272.
27. Gao, Y.L.; Zhu, R.S.; Liu, C.Y.; Li, W.F.; Jiang, H.W.; Li, C.D.; Yao, B.C.; Hu, G.H.; Chen, Q.S. Constructing Molecular Identity for Soybean Varieties from Heilongjiang Province, China. *Acta Agron. Sin.* **2009**, *35*, 211–218. [[CrossRef](#)]
28. Lee, G.Y.; So, Y.S.; Kim, H.S.; Hong, J.H.; Jang, E.K.; Lee, K.H.; Yun, G.S.; Hwang, S.G. Variation of Growth and Yield Component Character and Varietal Classification by Multivariate Analysis in Korean Soybean Varieties. *Korean J. Int. Agric.* **2015**, *27*, 348–357. [[CrossRef](#)]

29. Lee, C.; Choi, M.S.; Kim, H.T.; Yun, H.T.; Lee, B.; Chung, Y.S.; Kim, R.W.; Choi, H.K. Soybean [*Glycine max* (L.) Merrill]: Importance as A Crop and Pedigree Reconstruction of Korean Varieties. *Plant Breed. Biotech.* **2015**, *3*, 179–196. [[CrossRef](#)]
30. Schmutz, J.; Cannon, S.B.; Schlueter, J.; Ma, J.; Mitros, T.; Nelson, W.; Hyten, D.L.; Song, Q.; Thelen, J.J.; Cheng, J.; et al. Genome sequence of the palaeopolyploid soybean. *Nature* **2010**, *463*, 178–183. [[CrossRef](#)]
31. Song, Q.; Jia, G.; Zhu, Y.; Grant, D.; Nelson, R.T.; Hwang, E.Y.; Hyten, D.L.; Cregan, P.B. Abundance of SSR Motifs and Development of Candidate Polymorphic SSR Markers (BARCSOYSSR\_1.0) in Soybean. *Crop Sci.* **2010**, *50*, 1950–1960. [[CrossRef](#)]
32. Kim, Y.H.; Park, H.M.; Hwang, T.Y.; Lee, S.K.; Choi, M.S.; Jho, S.; Hwang, S.; Kim, H.M.; Lee, D.; Kim, B.C.; et al. Variation Block-Based Genomics Method for Crop Plants. *BMC Genom.* **2014**, *15*, 477–490. [[CrossRef](#)] [[PubMed](#)]
33. Sohn, H.B.; Kim, S.J.; Hwang, T.Y.; Park, H.M.; Lee, Y.Y.; Koo, B.C.; Kim, Y.H. Chromosome Reshuffling Patterns of Korean Soybean Cultivars Using Genome-Wide 202 InDel Markers. *Korean J. Breed. Sci.* **2017**, *49*, 213–223. [[CrossRef](#)]
34. Sohn, H.B.; Kim, S.J.; Hwang, T.Y.; Park, H.M.; Lee, Y.Y.; Markkandan, K.; Lee, D.; Lee, S.; Hong, S.Y.; Song, Y.H.; et al. Barcode System for Genetic Identification of Soybean [*Glycine max* (L.) Merrill] Cultivars Using InDel Markers Specific to Dense Variation Blocks. *Front. Plant Sci.* **2017**, *8*, 520–531. [[CrossRef](#)] [[PubMed](#)]
35. Sohn, H.B.; Song, Y.H.; Kim, S.J.; Hong, S.Y.; Kim, K.D.; Koo, B.C.; Kim, Y.H. Identification and Chromosomal Reshuffling Patterns of Soybean Cultivars Bred in Gangwon-do using 202 InDel Markers Specific to Variation Blocks. *Korean J. Breed. Sci.* **2018**, *50*, 396–405. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).