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Genetic Diversity of Sweet Potato (*Ipomoea batatas* L. Lam) Germplasms Collected Worldwide Using Chloroplast SSR Markers

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Abstract: Sweet potato (*Ipomoea batatas* L. Lam) is an important food crop widely cultivated in the world. In this study, nine chloroplast simple sequence repeat (cpSSR) markers were used to analyze the genetic diversity and relationships of 558 sweet potato accessions in the germplasm collection of the National Agrobiodiversity Center (NAC). Eight of the nine cpSSR showed polymorphisms, while Ibcp31 did not. The number of alleles per locus ranged from two to four. In general, the Shannon index for each cpSSR ranged from 0.280 to 1.123 and the diversity indices and unbiased diversity ranged from 0.148 to 0.626, and 0.210 to 0.627, respectively. Results of the median-joining network showed 33 chlorotypes in 558 sweet potato accessions. In factor analysis, 558 sweet potato accessions were divided into four clusters, with clusters I and II composed only of the sweet potato accessions from Korea, Japan, Taiwan, and the USA. The results of this study confirmed that the genetic diversity of the female parents of sweet potato accessions conserved at the NAC is low and therefore more sweet potato accessions need to be collected. These results will help to establish an efficient management plan for sweet potato genetic germplasms at the NAC.

Keywords: chloroplast; *Ipomoea batatas*; simple sequence repeat; sweet potato; plant germplasm

1. Introduction

Plant genetic resource, one of the most essential natural resources, has been a research topic resulting in major advancement in the field [1]. Gene banks are concerned with the maintenance of crop resource genetic variations, and plant genetic resource conservation is now receiving greater attention [1,2]. In order to establish effective and efficient conservation practices for plant genetic resources, understanding the genetic diversity between and within population is important [3].

Sweet potato (*Ipomoea batatas* L. Lam.) is a vegetative propagation crop that belongs to the family Convolvulaceae [4]. The origin of sweet potato is either the Central or South America [5]. Sweet potato is attractive to resource-poor farmers because they have the highest rate of production per unit area/time [6]. It also has a short growth period and is easily propagated and grown with good production in various climates and farming systems [7].

Molecular techniques have been widely adopted as powerful tools for germplasm characterization, cultivar identification, phylogenetic studies, and diversity analysis in many crop plants [8]. In sweet potato studies, molecular markers have been used to evaluate the phylogenetics and germplasm to study the origin of sweet potato and its dissemination [9]. Random amplified polymorphic DNA

(RAPD), DNA amplification fingerprinting (DAF), and simple sequence repeat (SSR) markers have been used for the estimation of genetic diversity and genetic relationships [6,8,10,11]. Previous studies have explained the genetic diversity and origin of sweet potato landraces in Mexico, Peru, and New Guinea using chloroplast and nuclear SSR [12,13].

During early genome sequencing projects, chloroplast DNAs (cpDNAs) were of interest because of their small size [14]. The chloroplast (CP) genome was observed in an extremely conserved manner not only in terms of gene numbers, but also in their arrangement [15]. CpSSR derived from the chloroplast genome represents ideal complementary molecular tools to nuclear genetic markers. This is because the SSR loci in the chloroplast genome are often distributed throughout the noncoding regions where higher sequence variations exist, shown to be due to low evolutionary rate and an almost nonexistent recombination rate in chloroplast DNA [16–18]. Therefore, cpSSR markers can be used to investigate population genetics and biogeography, and unravel the genetic relationships of closely related species [19].

During the 18th century, sweet potatoes were brought from Japan to various parts of Korea as a famine-relief crop [20]. At present, about 700 sweet potato germplasms have been collected worldwide at the National Agrobiodiversity Center (NAC) at the Rural Development Administration in Korea. However, analysis of genetic diversity in the preserved sweet potato accessions in NAC is lacking. Therefore, it is necessary to learn the genetic relationship of the sweet potato accessions to efficiently manage sweet potato germplasms. In this study, 558 sweet potato accessions conserved at the NAC were analyzed using nine cpSSRs to evaluate the genetic diversity and determine the appropriate panel of sweet potato germplasm for sweet potato improvement and conservation.

2. Materials and Methods

2.1. Plant Materials

Fresh leaves were randomly collected from five to ten individuals selected from 558 sweet potato (*Ipomoea Batatas* (L.) Lam) accessions conserved at the National Agrobiodiversity Center (NAC) in South Korea. The 558 sweet potato accessions were collected from ten countries including 190 accessions from South Korea (KOR), 123 from Japan (JPN), 73 from Taiwan (TWN), 50 from Peru (PER), 43 from China (CHN), 30 from Indonesia (IDN), 25 from the United States (USA), nine from the Philippines (PHL), nine from New Zealand (NZE), and six from North Korea (PRK) (Table 1 and Table S1).

Table 1. Number of sweet potato accessions in this study.

	Unknown	Breeding Line	Cultivar	Landrace	Total
CHN [1]	11	2	29	1	43
IDN		3	18	9	30
JPN	15	22	79	7	123
KOR		125	48	17	190
NZE			9		9
PER			50		50
PHL	1		8		9
PRK	2		3	1	6
TWN	31	32	10		73
USA			22	3	25
Total	60	184	276	38	558

[1] CHN, China; IND, Indonesia; JPN, Japan; KOR, South Korea; NZE, New Zealand; PER, Peru; PHL, Philippines; PRK, North Korea; TWN, Taiwan; USA, United States.

2.2. DNA Extraction

DNA was extracted from 100 mg of freeze-dried leaves in each sweet potato accession. The DNeasy plant mini kit (Qiagen, Hilden, Germany) was used for the extractions. The DNA quality and quantity

of each sample were determined by electrophoresis in 1% (w/v) agarose gels and spectrophotometry (Epoch, BioTek, Winooski, VT, USA). The extracted DNA was diluted to 30 ng/uL and was stored at −20 °C until further PCR amplification.

2.3. Chloroplast SSR Genotyping

For chloroplast SSR analysis, a total of nine cpSSRs were fluorescently labelled (6-FAM, HEX, and NED) and used for detection of the amplification products (Table 2). The PCR reactions were carried out in a 25 uL mixture containing 30 ng template DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.5 um of each primer, and 1 U Taq polymerase (Inclone, Seongnam, Korea). The amplification was performed with cycling conditions of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. Each amplicon was resolved on an ABI Prism 3500 DNA sequencer (ABI3500, Thermo Fisher Scientific Inc., Wilmington, DE, USA) and was scored using Gene Mapper Software (Version 4.0, Thermo Fisher Scientific Inc., Wilmington, DE, USA)

Table 2. Nine cpSSR markers used in this study.

SSR Marker	Primer Sequences (5′→3′)	Dye
ccmp2	F: GATCCCGGACGTAATCCTG R: ATCGTACCGAGGGTTCGAAT	6-FAM
NTCP18	F: CTGTTCTTTCCATGACCCCTC R: CCACCTAGCCAAGCCAGA	HEX
NTCP28	F: TCCAATGGCTTTGGCTA R: AGAAACGAAGGAACCCAC	NED
NTCP26	F: GCAATTGCAATGGCTTCTTTA R: TTTATGTTCGGTGGAAATCACA	6-FAM
Ibcp5	F: GCTCTCACGCTCAATTACTTA R: ATGCTTAATTGACGACCTGT	HEX
Ibcp8	F: AATAAGTACTTGGCCGTGAA R: CGATTCAAGTAGGCAAAGAG	NED
Ibcp10	F: ATATAAGGGGCCATTTTAGG R: ACGATAGAGGAGAAGGTTCC	6-FAM
ibcp4	F: ATCCTGGACGTGAAGAATAA R: GATGGCTGAGTGGACTAAAG	HEX
ibcp31	F: AACGGATTTCTCCAATGTA R: ACCTCACCGTTTCAGAAGTA	NED

2.4. Data Analysis

The number of observed alleles (Na) for each cpSSR locus was counted for all sweet potato accessions. The effective number of alleles (Ne = 1/(Sum piˆ2)), the Shannon index ((I = −1 × Sum (pi × Ln (pi)), the frequency of the I allele), the diversity index (h = 1 − Sum piˆ2), and the unbiased diversity (uh = (N/(N − 1)) × h) were calculated using GenAlEx 6.5 [21]. Genetic differentiation between the populations was determined using PhiPT, a measure that allows intra-individual variation to be suppressed and is therefore ideal for comparing cpSSR data with 999 permutations using GenAlEx. Estimates of gene flow (Nm, number of migrants per generation = [(1/PhiPT) − 1]/2) were also calculated by GenAlEx. Analysis of molecular variance (AMOVA), among and within the subpopulations (assigned by the median-joining network), was performed in GenAlEx. To calculate distances based on the cpSSR data, DARwin v.6.0 was employed to generate genetic distance matrix, which were then used to perform factor analysis [22]. To examine the relationship between the 558 sweet potato accessions, a median-joining network was generated using *poppr* packages on R packages [23] and visualized using Network 5 [24].

3. Results

A total of 21 alleles were detected in eight polymorphic cpSSR loci among the 558 sweet potato germplasms. As shown in Table 3, the Na ranged from two to four and the Ne was calculated to range from 1.174 to 2.675. Shannon's information (I) for each cpSSR ranged from 0.280 to 1.123 and the diversity indices (h) and uh ranged from 0.148 to 0.626 and 0.148 to 0.627, respectively.

Table 3. The genetic diversity parameters of nine cpSSR markers in 558 sweet potato germplasms.

SSR marker	Na [1]	Ne	I	h	uh
Ibcp10	3	2.216	0.933	0.549	0.550
Ibcp31	1		monomorphic		
Ibcp4	3	2.050	0.829	0.512	0.513
Ibcp5	2	1.266	0.365	0.210	0.210
Ibcp8	2	1.918	0.672	0.479	0.480
NTCP18	2	1.174	0.280	0.148	0.148
NTCP26	4	2.675	1.123	0.626	0.627
NTCP28	2	1.812	0.640	0.448	0.449
ccmp2	3	2.072	0.839	0.517	0.518
Mean	2.4 ± 0.3	1.798 ± 0.18	0.631 ± 0.12	0.388 ± 0.07	0.388 ± 0.07

[1] Na = No. of alleles; Ne = No. of effective alleles; I = Shannon's information index; h = Diversity; uh = unbiased diversity.

The diversity indices among the ten countries were calculated to be Ne = 1.52, I = 0.44, and h = 0.29 (Table 4). The Na ranged from 1.44 (PRK) to 2.22 (JPN) and the Ne was calculated to be 1.25 (PRK) to 1.77 (TWN). Shannon's information (I) ranged from 0.23 (PRK) to 0.61 (JPN). The diversity indices (h) and uh were 0.15 (PRK) to 0.39 (JPN) and 0.19 (PRK) to 0.39 (JPN), respectively.

Table 4. The genetic diversity parameters of ten origins in the 558 sweet potato germplasms using eight cpSSRs.

Countries	Na [1]	Ne	I	h	uh
JPN	2.22 ± 0.28	1.75 ± 0.16	0.61 ± 0.10	0.39 ± 0.06	0.39 ± 0.06
KOR	2.11 ± 0.20	1.64 ± 0.14	0.54 ± 0.10	0.35± 0.07	0.35 ± 0.07
TWN	2.11 ± 0.20	1.77 ± 0.18	0.56 ± 0.12	0.37 ± 0.08	0.38 ± 0.08
USA	2.11 ± 0.20	1.51 ± 0.08	0.51 ± 0.08	0.31 ± 0.05	0.33 ± 0.05
CHN	2.11 ± 0.20	1.45 ± 0.12	0.44 ± 0.10	0.27 ± 0.06	0.28 ± 0.07
PHL	1.67 ± 0.17	1.41 ± 0.13	0.36 ± 0.10	0.24 ± 0.07	0.27 ± 0.08
PER	1.89 ± 0.20	1.56 ± 0.16	0.44 ± 0.11	0.30 ± 0.08	0.30 ± 0.08
PRK	1.44 ± 0.18	1.25 ± 0.12	0.23 ± 0.09	0.15 ± 0.06	0.19 ± 0.08
IDN	1.78 ± 0.22	1.55 ± 0.17	0.42 ± 0.12	0.29 ± 0.08	0.30 ± 0.08
NZE	1.67 ± 0.24	1.30 ± 0.10	0.31 ± 0.10	0.19 ± 0.06	0.22 ± 0.07
Mean	1.91 ± 0.07	1.52 ± 0.04	0.44 ± 0.03	0.29 ± 0.02	0.30 ± 0.02

[1] Na = No. of different alleles; Ne = No. of effective alleles; I = Shannon's information index; h = Diversity; uh = unbiased diversity.

To improve understanding of the genetic relationships between different chloroplast haplotypes, a network approach was used (Figure 1). A total of 77 different chloroplast haplotypes in sweet potato germplasms were identified according to the results of the median-joining network (Table 5). Forty-four of the 77 different chloroplast haplotypes were found only once. Among the 33 chloroplast haplotypes with two or more sweet potato accessions, H1 and H2 were clearly dominant and were distributed over all areas, except for North Korea and New Zealand. The number of chloroplast haplotypes per country ranged from 3 to 41 with an average of fifteen. The diversity of chloroplast haplotypes ranged from 0.216 to 0.833 with an average of 0.37. Although the number of sweet potato accessions in North Korea was the lowest (six), the diversity of chloroplast haplotypes was the highest (five types, 83.3%)

among the countries studied. South Korea showed the lowest diversity at 21.6%, displaying only 27 chloroplast haplotypes out of 190 sweet potato accessions.

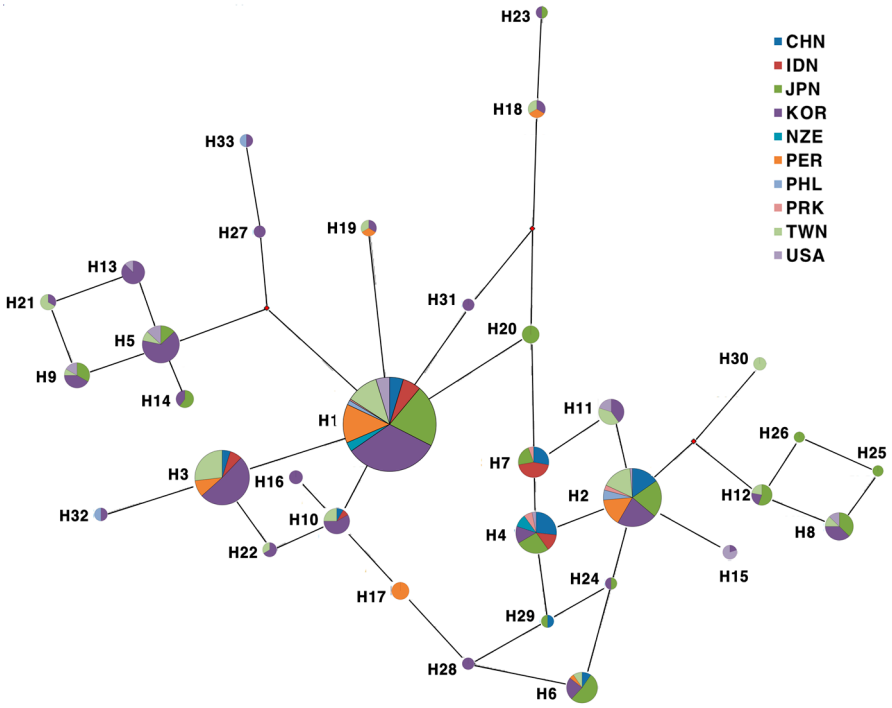

Figure 1. Median-joining network of eight cpSSR haplotypes. Circle size is proportional to the number of accessions that carry this chloroplast haplotype.

Table 5. Geographical distribution of cpSSR haplotypes.

	KOR	JPN	TWN	USA	CHN	PER	IDN	PRK	PHL	NZE
N [1]	190	123	73	25	43	50	30	6	9	9
H1	62	40	21	9	9	25	12	1	3	6
H2	16	15	12	1	11	11		2	4	
H3	21		11		2	4	3			
H4	4	8		1	8		4	2		3
H5	15	3	2	3						
H6	5	11	2		2	1				
H7		4			5		8	1		
H8	6	6	2	2						
H9	5	4	1	2						
H10	7		3		1		1			
H11	4		4	2						
H12	2	5	2							
H13	7			1						
H14	2	3								
H15	1			4						
H16	4									
H17						3				
H18	1		1			1				
H19	1		1			1				
H20		3								
H21	1		2							
H22	2		1							
H23	1	1								
H24	1	1								
H25		2								

Table 5. *Cont.*

	KOR	JPN	TWN	USA	CHN	PER	IDN	PRK	PHL	NZE
H26		2								
H27	2									
H28	2									
H29		1			1					
H30			2							
H31	2									
H32	1								1	
H33	1								1	
PH	14	14	6		4	4		2		
H	41	31	22	5	13	12	5	8	3	10
DH	0.216	0.252	0.301	0.833	0.302	0.24	0.556	0.267	0.333	0.40

[1] N, Number of accessions; PH, private chloroplast haplotype; H, number of distinct chloroplast haplotypes; DH, diversity of chloroplast haplotype.

Factorial analysis (FA) was used to investigate the genetic relationship among the chloroplast haplotypes of the 558 sweet potato accessions. The sweet potato chloroplast haplotypes formed four main clusters defined by the first axis (Figure 2A), which explained 54.1% of the total variances, while the second axis explained 9.5% of the total variances. Cluster I contained 51 sweet potato accessions with five chloroplast haplotypes (H5, H9, H13, H14, and H21). Cluster II consisted of 29 accessions and showed four chloroplast haplotypes, H8, H12, H25, and H26. Cluster III had the most sweet potato accessions (268 accessions) and chloroplast haplotypes (H1, H3, H10, H16, H17, H18, H19, H20, H22, H27, H31, H32, and H33) than the other clusters. Cluster IV had 168 accessions and 11 chloroplast haplotypes (H2, H6, H11, H15, H23, H24, H30, H4, H7, H28, and H29). Among the four clusters, clusters III and IV were distributed across all ten collected countries, while I and II were found only in four countries: Korea, Japan, Taiwan, and the USA (Figure 2B).

The AMOVA analysis performed on the whole sweet potato germplasms indicated 88% total genetic diversity within the populations and 12% total genetic diversity among the populations (Table 6). The genetic differences (PhiPT) among the populations were estimated by AMOVA to be 0.125. The gene flow (genetically effective migration rate, Nm) among the populations was indirectly calculated from the PhiPT as 3.513. In addition, based on factorial analysis, AMOVA of the four clusters was conducted. The analysis revealed that 9, 36, and 4% of the total genetic diversity of the populations was attributable to clusters I, III, and IV, respectively, while cluster II was not significant ($p > 0.05$). The PhiPT of cluster I, III, and IV were 0.093, 0.361, and 0.042 and the gene flow of each cluster was 4.870, 0.885, and 11.370, respectively.

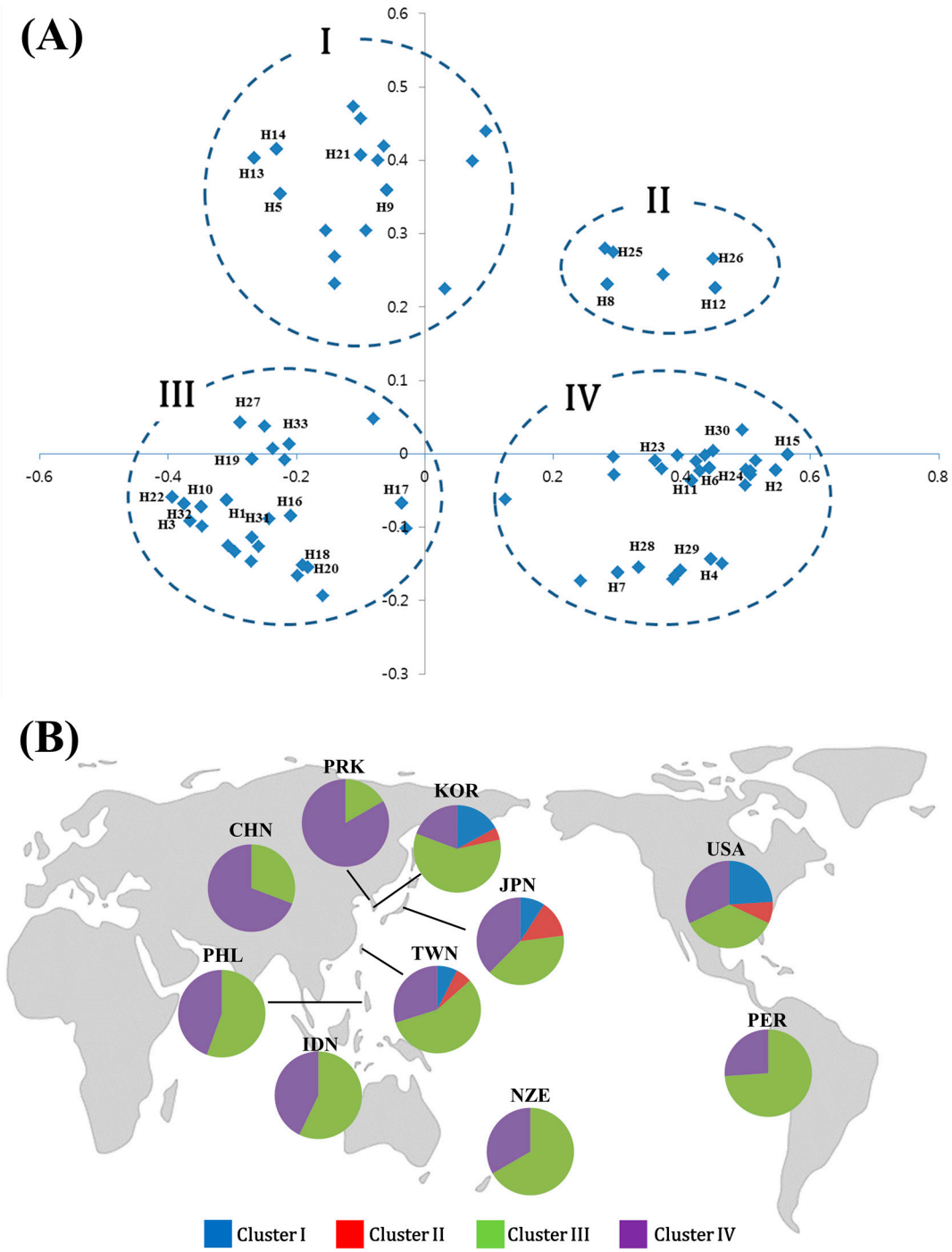

Figure 2. (**A**) Factorial analysis of the 8 cpSSR haplotypes. H, chloroplast haplotypes based on the median-joining network. (**B**) Original countries of sweet potato accessions and geographic distributions of the chloroplast haplotypes found. Pie charts on the map represent the chlorotype composition of the accessions and the colors in each chart represent the chlorotype indicated by factorial analysis.

Table 6. Analysis of molecular variance (AMOVA) of sweet potato germplasms.

Source of Variation	df	SS	MS	Est. Var.	%	PhiPT	Sig.	Nm
Whole data set								
Among Populations	9	120.09	13.34	0.236	12%	0.125	<0.001	3.513
Within Populations	548	908.38	1.66	1.658	88%			
Total	557	1028.47		1.894	100%			
I								
Among Populations	3	2.73	0.91	0.046	9%	0.093	<0.05	4.870
Within Populations	47	21.00	0.45	0.447	91%			
Total	50	23.73		0.493	100%			
II								
Among Populations	3	1.94	0.65	0.051	13%	0.134	ns	3.244
Within Populations	25	8.33	0.33	0.333	87%			
Total	28	10.28		0.385	100%			
III								
Among Populations	9	42.48	4.72	0.273	36%	0.361	<0.001	0.885
Within Populations	156	75.40	0.48	0.483	64%			
Total	165	117.89		0.756	100%			
IV								
Among Populations	8	5.96	0.74	0.015	4%	0.042	<0.05	11.370
Within Populations	258	89.72	0.35	0.348	96%			
Total	266	95.68		0.363	100%			

4. Discussion

In this study, the genetic diversity of 558 sweet potato germplasms conserved in the Genebank was profiled using nine cpSSRs. The results confirmed that the sweet potato germplasms showed various genetic variations depending on the country of collection. Previous studies have been performed to analyze the genetic diversity in sweet potato using molecular markers such as AFLP, RAPD, and SSR [6,8,10,11,25]. In addition, the origin and genetic diversity of sweet potato germplasms have been explained using nuclear SSRs and cpSSRs [12,13]. These methods, non-recombinant and uniparentally inherited nature, are widely used for evolutionary and phylogenetic studies as they have been demonstrated to be effective indicators of the genetic structure of a population [26,27].

In this study, 92.4% of the accessions were included in the breeding lines or cultivars among the 558 sweet potato germplasms. Many other genetic resource centers showed a similar composition of preserved sweet potato accessions. The International Potato Center (CIP) reported that 93.3% of the sweet potato accessions in 36 international germplasm centers were cultivars or breeding lines and that only seven centers were preserving the wild type [28]. There appears to be a higher frequency of breeding line or cultivars due to the breeding system of sweet potatoes. In general, numerous seedlings of posterities resulting from crossing elite parent sweet potatoes were screened for desirable traits and the best were used, with or without the best parents. The undesirable genotypes were discarded through a selection process that concentrated on eliminating the poorest, rather than on selecting the best genotypes [29]. In Japan, they harvested approximately 50,000 seeds from cross combinations and selected approximately 3000 individual seedling plants. After that, they conducted various tests for line selection and generated cultivars or maintained elite breeding lines [4]. For example, cv. 'Beniazuma' was released by the Institute of Crop Science, NARO (NICS) in 1984 and is the progeny of a cross between 'Kanto No. 85' and 'Koganesenga' [30]. Similarly, 'Quick Sweet' was released by the NICS in 2002 and is the progeny of a cross between 'Beniazuma' and Kyushu No. 30' [31]. Additionally, 'Aikomachi' was released by the NICS in 2012 and was derived from a cross between 'Quick Sweet' and 'Kankei107' [32]. These three cultivars may show the same patterns in cpSSRs profiling because they have the same maternal pedigree, even though they have different cross combinations. The Korean method is similar to the method of Japanese sweet potato breeding. For example, Korean sweet potato cultivars (cvs), 'Pungmi', Yeonmi', 'Sinyulmi', and 'Gogeonmi' were derived from cv. 'Seonmi' while

cvs. 'Seonmi', 'Jinmi', and 'Eunmi' were derived from cv. 'Hwangmi'. Seven Korean sweet potato cultivars ('Pungmi', Yeonmi', 'Sinyulmi', 'Gogeonmi' 'Seonmi', 'Jinmi', and 'Eunmi') have the same cpSSRs patterns because they share the same maternal pedigree, even though they have different cross combinations. Due to the frequent use and preservation of these elite accessions, it is possible that conserved sweet potato germplasms such as the breeding lines or cultivars showed the same patterns of cpSSRs.

Among the 558 sweet potato accessions collected from ten countries, sweet potato germplasms from Japan, Korea, Taiwan, and the USA showed four types while other countries had only two types (Figure 2). The Korean sweet potato germplasm collection was started from Japanese varieties. In 1906, 1923, and 1934, Japanese varieties Genki, Shirofuku, and Okinawa No. 100 were introduced and cultivated throughout Korea, respectively. The collection of sweet potato germplasms in Korea began in 1973, led by the international agricultural institutes, International Potato Center (CIP) and World Vegetable Center (AVRDC). In Korean landraces, superior sweet potato lines were selected during breeding programs processes. Although the collection of sweet potatoes was not smooth due to problems with the Peruvian government's approval by CIP, sweet potato germplasms were obtained from AVRDC, USDA, and Japan [33].

A previous study reported that variation in the maternally-inherited plant genomes affected both type weight and root weight in sweet potatoes [34]. In addition, it was also mentioned that the selection of Nancy Hall and Tainung 27 as female parents significantly affected the two traits. Although the sweet potato germplasms conserved at the Korea Genebank represent approximately 700 accessions, the results of this study showed that a variety of female parents was lacking. In fact, the sweet potato germplasms contained in the Korea Genebank are almost all cultivars or breeding lines without their wild type relatives [35]. Since 1955, Mexico and the United States have collected many wild plants related to sweet potatoes to study sweet potato's phylogeny and the utilization of wild plants in sweet potato breeding [36]. Wild relatives have provided breeders with genes for pest and disease resistance, abiotic stress tolerance, and quality traits [37]. The results of this study confirmed that sweet potato germplasms conserved in the Korea Genebank need a greater variety of female parents. Despite this, analysis of the genetic diversity of nuclear DNA in sweet potato germplasms should still be conducted. Additional collection and conservation of various female parents and wild relatives of sweet potatoes are very important to improve the quality of sweet potato germplasm and satisfy the demand of breeders.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/11/752/s1, Table S1: List of 558 sweet potato germplasms.

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Conflicts of Interest: The authors declare no conflicts of interest.

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