

Genetic Diversity and Population Structure of Peanut (*Arachis hypogaea* L.) Accessions from Five Different Origins

Kunyan Zou¹, Ki-Seung Kim², Daewoong Lee³, and Tae-Hwan Jun^{4,5,†}

ABSTRACT Peanut is an allotetraploid derived from a single recent polyploidization. Polyploidization has been reported to have caused significant loss in genetic diversity during the domestication of cultivated peanuts. Single nucleotide polymorphism (SNP)-based markers such as cleaved amplified polymorphic sequences (CAPS) derived from next-generation sequencing (NGS) have been developed and widely applied for breeding and genetic research in peanuts. This study aimed to identify the genetic diversity and population structure using 30 CAPS markers and 96 peanut accessions from five different origins. High genetic dissimilarities were detected between the accessions from Korea and those from the other three South American origins generally regarded as the origin of peanuts, while the accessions from Brazil and Argentina presented the lowest genetic dissimilarity. Based on the results of the present study, accessions from Korea have unique genetic variation compared to those from other countries, while accessions from the other four origins are closely related. Our study identified the genetic differentiation in 96 peanut accessions from five different origins, and this study also showed the successful application of SNP information derived from re-sequencing based on NGS technology.

Keywords : CAPS marker, genetic differentiation, genetic diversity, peanut, population structure

Peanut (*Arachis hypogaea* L.) is widely cultivated from tropical to temperate regions such as Africa, America and Asia with an annual production of about 42.4 million tons (FAO, 2020). It is also one of the main sources of cooking oil in the world, which contains 25% protein and 50% of oil. Peanut has a significant role in sustainable agriculture in terms of global food security and nutrition, fuel and energy, sustainable fertilization, and enhanced agricultural productivity as a rotation crop (Feng *et al.*, 2012).

Cultivated peanut is allotetraploid ($2n = 4 \times = 40$, AABB) with a genome size of 2800 Mb/1C and the genome composition of cultivated peanut was known to have derived from a recent hybridization of *A. duranensis* (A subgenome) and *A. ipaensis* (B subgenome) (Smartt *et al.*, 1978; Seijo *et al.*, 2007; Robledo *et al.*, 2009; Bertioli *et al.*, 2016). The genetic diversity of

cultivated peanut is extremely low because of the single recent polyploidization during domestication (Kim *et al.*, 2017). Peanut subgenomes show a high similarity (Kottapalli *et al.*, 2007; Khara *et al.*, 2013) with an estimated repetition rate of 64%, which makes the assembly of peanut genome sequences extremely difficult (Dhillon *et al.*, 1980; Temsch & Greilhuber, 2000; Bertioli *et al.*, 2016). The genome sequences of the diploid ancestors (*A. duranensis* and *A. ipaensis*) of cultivated peanut were reported in 2016, which became the basis for understanding the genome of cultivated peanut (Ren *et al.*, 2011). Recently, the reference sequence of cultivated peanut allotetraploid *A. hypogaea* genome was reported in 2019 and compared with the related diploid *A. duranensis* and *A. ipaensis* genomes. A total of 39,888 A subgenome genes and 41,526 B subgenome genes were annotated in the allotetraploid subgenomes (Chen *et al.*,

¹PhD Student, Department of Plant Bioscience, Pusan National University, Miryang 50463, Korea

²Senior Researcher, FarmHannong, Ltd., Daejeon 34115, Korea

³MSc Student, Department of Plant Bioscience, Pusan National University, Miryang 50463, Korea

⁴Professor, Department of Plant Bioscience, Pusan National University, Miryang 50463, Korea

⁵Professor, Life and Industry Convergence Research Institute, Pusan National University, Miryang 50463, Korea

†Corresponding author: Tae-Hwan Jun; (Phone) +82-55-350-5507; (E-mail) thjun76@pusan.ac.kr

<Received 2 September, 2020; Accepted 12 September, 2020>

2019).

Recently, next generation sequencing (NGS) technology has made significant progress and the sequencing cost has dropped sharply (Kim *et al.*, 2017). In addition, the accuracy and productivity of sequencing data have indeed improved innovatively. In particular, NGS technologies such as *De novo* assembly and resequencing based on a variety of bioinformatics methods have enabled the production of large numbers of single nucleotide polymorphism (SNP) and simple sequence repeats (SSR) in complex genomes (Yang *et al.*, 2012; Lee *et al.*, 2015; Bertoli *et al.*, 2016; Kang *et al.*, 2016). Using NGS technology, high throughput genotyping was conducted by using double-digest restriction-site associated DNA sequencing (ddRADseq), a total of 14,663 SNPs were developed and used for the construction of a genetic linkage map in peanut cultivars (Zhou *et al.*, 2014). Numerous SNP and CAPS markers have been developed from the re-sequencing of the two Korean peanut cultivars “K-OL” and “Pungan”, which means that the molecular marker information can provide valuable guidance and information for peanut breeding program (Kim *et al.*, 2017).

Cleaved amplified polymorphic sequence (CAPS) is the combination of PCR amplification and restriction enzyme analysis, SNP occurs within the recognition site of a restriction enzyme. The digestion of PCR products can be carried out in laboratory with separation of the fragments in agarose gel. Because of the convenience of analysis, development of SNP-based markers such as CAPS has been widely carried out followed by NGS analysis, and the developed markers have been used to figure out genetic diversity or population structure in crops (Rasheed *et al.*, 2017; Wang *et al.*, 2017).

In this study, we aimed to evaluate genetic diversity and population structure in 96 peanut accessions derived from five different origins using the CAPS markers developed from re-sequencing of two Korean peanut cultivars

MATERIALS AND METHODS

Plant material and DNA extraction

A total of 96 peanut accessions obtained from the National Agrobiodiversity Center, Jeonju, Republic of Korea were used for the present study (Table 1). Ninety-six accessions were originally donated from five countries; two accessions from Peru (PRE), thirteen accessions from China (CHN), fifteen accessions

from Argentina (ARG), 17 accessions from Brazil (BRA), and 49 accessions from Korea (KOR). In 2017, the accessions were planted in a greenhouse at Pusan National University, Miryang, Republic of Korea. A young leaf from each individual accession was collected to extract genomic DNA. Genomic DNA was extracted for each accession with the CetyltrimethylAmmonium Bromide (CTAB) protocol (Saghai-Marooof *et al.*, 1984) with minor modifications. The quality and quantity of the extracted DNA were measured with a NanoDrop ND-1000 (Thermo Fisher Scientific Inc., USA) and electrophoresis on a 1% agarose gel. Final concentration of each DNA sample was adjusted to 30 ng/ μ l.

Analysis of the CAPS markers

A total 30 CAPS markers were used to evaluate genetic diversity and population structure of the peanut accessions in the present study (Supplementary Table S1). The CAPS markers were derived from thirteen different chromosomes (A01, A03, A05, A06, A07, A08; B01, B03, B04, B06, B07, B08). It has been confirmed that 28 of the CAPSs were in intergenic regions and two CAPSs were in coding regions in peanut genome (Kim *et al.*, 2017).

Polymerase chain reaction (PCR) amplifications were conducted in 20 μ L reactions containing 60 ng of template DNA, 5nM mixed Primer, 1X reaction buffer, 10mM dNTP, and 1.0 unit of *Taq* DNA polymerase (Gen-Script USA Inc., Piscataway, N.J., USA). PCR product was digested with enzyme (*AseI*, *DraI*, *HpaII*, *MseI*, *MspI*, *PstI*, *Taq. I*) (New England Biolabs, USA; Enzynomics, Republic of Korea) and incubated at T-100 thermal cycler (BIO-RAD, USA) using optimum enzyme cutting temperature for 1 h. PCR products and the restriction enzyme-digested PCR products were resolved on 1.5% agarose gels (Promega, USA) to detect the polymorphism.

Data analysis

UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram was constructed using the MEGA 4 (Tamura *et al.*, 2007). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the accessions analyzed, and branches corresponding to partitions reproduced in less than 42 % bootstrap replicates collapsed (Felsenstein, 1985). The evolutionary distances were computed among the 96 accessions by using the Maximum Composite

Table 1. Information on the 96 peanut accessions used in the study.

No	IT no ^a	Origin ^b	Seed color ^c	Variety ^d	Growth habit ^e	100 sw ^f	Seed size ^g
1	IT030843	KOR	Tan	Landrace	Half erect	51.50	Middle
2	IT030923	KOR	Red	Breeding line	Erect	80.26	Middle
3	IT030847	KOR	Tan	Landrace	Half erect	66.20	Middle
4	IT030848	KOR	Tan	Landrace	Erect	79.78	Middle
5	IT030851	KOR	Tan	Landrace	Half erect	77.60	Middle
6	IT030858	KOR	Tan	Landrace	Half erect	64.40	Middle
7	IT030859	KOR	Tan	Landrace	Erect	81.96	Big
8	IT030862	KOR	Tan	Landrace	Erect	76.88	Middle
9	IT030867	KOR	Tan	Landrace	Half erect	64.10	Middle
10	IT030930	KOR	Tan	Breeding line	Erect	79.92	Middle
11	IT030871	KOR	Tan	Landrace	Half erect	55.80	Middle
12	IT030873	KOR	Tan	Landrace	Spreading	70.10	Middle
13	IT030880	KOR	Tan	Landrace	Half erect	63.56	Middle
14	IT030881	KOR	Tan	Landrace	Half erect	59.00	Middle
15	IT030882	KOR	Red	Landrace	Erect	79.34	Middle
16	IT030886	KOR	Tan	Landrace	Erect	35.84	Small
17	IT030887	KOR	Tan	Landrace	Erect	42.44	Small
18	IT030888	KOR	Tan	Landrace	Erect	39.80	Small
19	IT030953	KOR	Tan	Landrace	Erect	41.30	Small
20	IT030957	KOR	Tan	Breeding line	Half erect	53.90	Middle
21	IT110193	BRA	Tan	Unknown	Half erect	29.80	Small
22	IT110214	KOR	Tan	Breeding line	Half erect	70.00	Middle
23	IT110222	KOR	Tan	Cultivar	Half erect	67.60	Middle
24	IT110224	KOR	Tan	Cultivar	Half erect	50.40	Middle
25	IT110233	ARG	Tan	Unknown	Erect	27.70	Small
26	IT110243	KOR	Tan	Landrace	Erect	73.06	Middle
27	IT110255	KOR	Tan	Landrace	Half erect	46.00	Small
28	IT171378	KOR	Tan	Breeding line	Erect	94.98	Big
29	IT172455	ARG	Red	Unknown	Bunch	47.05	Middle
30	IT172482	PER	Purple	Unknown	-	58.10	Middle
31	IT172547	KOR	Light brown	Breeding line	Erect	103.98	Big
32	IT172656	KOR	Light brown	Landrace	Erect	85.30	Big
33	IT172812	KOR	Red	Cultivar	Erect	101.74	Big
34	IT181768	KOR	Light brown	Breeding line	Erect	94.03	Big
35	IT030933	KOR	Tan	Breeding line	Erect	47.22	Small
36	IT184874	BRA	Red	Unknown	Erect	31.90	Small
37	IT184867	ARG	Red	Unknown	Spreading	32.50	Small
38	IT184889	BRA	Red	Unknown	Erect	29.8	Small
39	IT184896	BRA	Red	Unknown	Spreading and Bunch/ Erect	31.60	Small
40	IT184903	ARG	Purple	Unknown	Erect	30.50	Small
41	IT184910	BRA	Red	Unknown	Erect	35.10	Small
42	IT184938	CHN	Red	Unknown	-	40.20	Small
43	IT214799	KOR	Light brown	Cultivar	Erect	85.00	Middle
44	IT185030	CHN	Tan	Unknown	Erect	45.30	Small
45	IT185032	CHN	Red	Unknown	Erect	38.30	Small
46	IT185076	BRA	Red	Unknown	Erect	86.90	Big
47	IT191283	BRA	Red	Unknown	Half erect	50.30	Middle
48	IT191288	BRA	Red	Unknown	Half erect	33.60	Small
49	IT191293	ARG	Red	Unknown	Half erect	38.20	Small
50	IT191295	ARG	Red	Unknown	Half erect	30.00	Small

Table 1. Information on the 96 peanut accessions used in the study (Continued).

No	IT no ^a	Origin ^b	Seed color ^c	Variety ^d	Growth habit ^e	100 sw ^f	Seed size ^g
51	IT191315	BRA	Red	Unknown	Half erect	30.00	Small
52	IT191436	ARG	Red	Unknown	Half erect	28.70	Small
53	IT191437	ARG	Red	Unknown	Half erect	29.80	Small
54	IT191441	ARG	Red	Unknown	Erect	39.50	Small
55	IT191443	ARG	Red	Unknown	Erect	37.30	Small
56	IT184876	BRA	Red	Unknown	Erect	34.40	Small
57	IT191451	CHN	Red	Unknown	Half erect	32.80	Small
58	IT191456	BRA	Red	Unknown	Erect	27.00	Small
59	IT191504	ARG	Red	Unknown	Erect	28.40	Small
60	IT191513	BRA	Red	Unknown	Half erect	27.10	Small
61	IT191514	BRA	Red	Unknown	Half erect	26.40	Small
62	IT191524	BRA	Red	Unknown	Spreading	43.20	Small
63	IT191525	BRA	Red	Unknown	Half erect	31.70	Small
64	IT191528	BRA	Red	Unknown	Half erect	30.50	Small
65	IT191558	CHN	Tan	Unknown	Erect	46.00	Small
66	IT191587	CHN	Tan	Unknown	Erect	45.70	Small
67	IT196389	CHN	Red	Unknown	Erect	40.00	Small
68	IT207327	CHN	White	Unknown	Erect	76.00	Middle
69	IT213159	KOR	Purple	Cultivar	Erect	93.00	Big
70	IT213160	KOR	Light brown	Cultivar	Erect	83.00	Middle
71	IT213162	KOR	Tan	Cultivar	Erect	75.00	Middle
72	IT214781	KOR	Tan	Cultivar	Erect	103.00	Big
73	IT214787	KOR	Purple	Cultivar	Erect	99.00	Big
74	IT214789	KOR	Light brown	Cultivar	Erect	96.00	Big
75	IT214791	KOR	Tan	Cultivar	Half erect	103.00	Big
76	IT214792	KOR	Tan	Cultivar	Erect	108.00	Big
77	IT184886	BRA	Red	Unknown	Erect	32.60	Small
78	IT184973	CHN	Red	Unknown	Erect	64.48	Middle
79	IT214802	KOR	Brown	Cultivar	Half erect	118.00	Big
80	IT214803	KOR	Light brown	Cultivar	Erect	99.00	Big
81	IT191568	CHN	Red	Unknown	Erect	44.00	Small
82	IT214793	KOR	Tan	Cultivar	Erect	80.00	Big
83	IT214806	KOR	Tan	Cultivar	Erect	90.00	Big
84	IT221533	KOR	Brown	Cultivar	Erect	101.00	Big
85	IT221534	KOR	Brown	Cultivar	Erect	87.00	Big
86	IT221535	KOR	Purple	Cultivar	Erect	90.00	Big
87	IT267782	ARG	White	Unknown	Erect	33.00	Small
88	IT267783	ARG	Red	Unknown	Erect	25.00	Small
89	IT271343	CHN	Red	Landrace	-	67.00	Middle
90	IT214795	KOR	Tan	Cultivar	Erect	79.00	Big
91	IT271426	ARG	Purple	Unknown	Erect	58.00	Middle
92	IT271431	ARG	Purple	Unknown	Erect	30.00	Small
93	IT271463	PER	Light brown	Unknown	Erect	33.00	Small
94	IT271494	CHN	Red	Cultivar	-	62.00	Middle
95	IT271495	CHN	Purple	Cultivar	-	57.00	Middle
96	IT271499	KOR	Red	Landrace	Half erect	69.00	Middle

^{a-g}Information were obtained from Korean National Agrobiodiversity Center

^bARG, Argentina; BRA, Brazil; CHN, China; KOR, Korea; PER, Peru

^cSeed color of each accession was double-checked, and off-types were discarded. Tan indicates the Tannin color.

^eMiddle (51-70 seeds per oz), Small (71-90 seeds per oz), Big (36-50 seeds per oz).

Likelihood method (Tamura *et al.*, 2004).

The population structure of 96 peanut accessions was evaluated by Structure v2.3.4 software (https://web.stanford.edu/group/pritchardlab/structure_software/release_versions/v2.3.4/html/structure.html) under the admixture model. Models were tested for K-values ranging from 1 to 15, with 3 independent runs per K value. To make a decision for the optimum number of K, delta K (ΔK) method was used the software online “harvester structure” (Evanno *et al.*, 2005). Population structure and relationships were analyzed by principle coordinate analysis (PCoA) using software GenAlEx V6.503 (Peakall & Smouse, 2006).

For the estimation of genetic differentiation between subpopulations, the values of $F_{ST} > 0.25$ are taken to mean great differentiation between subpopulations; the range 0.15 to 0.25 indicates high differentiation; and the range 0.05 to 0.15 indicates moderate differentiation, while differentiation can negligible if $F_{ST} < 0.05$. Estimates of Genetic diversity indices

were calculated for each locus using GeneAIEx 6.503. The genetic differentiation between individual accessions was calculated using the F_{ST} to evaluate the reduction in genotypic heterozygosity (Grasso *et al.*, 2014).

RESULTS

Genetic diversity

The UPGMA tree placed the peanut accessions into two major clusters (Fig. 1), indicating that most accessions from Korea grouped in a cluster. The other cluster contained with accessions from other four origins and eight accessions collected from Korea show a small group in this cluster.

Accessions from BRA and ARG presented the F_{ST} values more than 0.25 compared to the KOR accessions (0.311 and 0.264, respectively) indicating the most significant differences between the accessions (Table 2). Accessions from KOR and

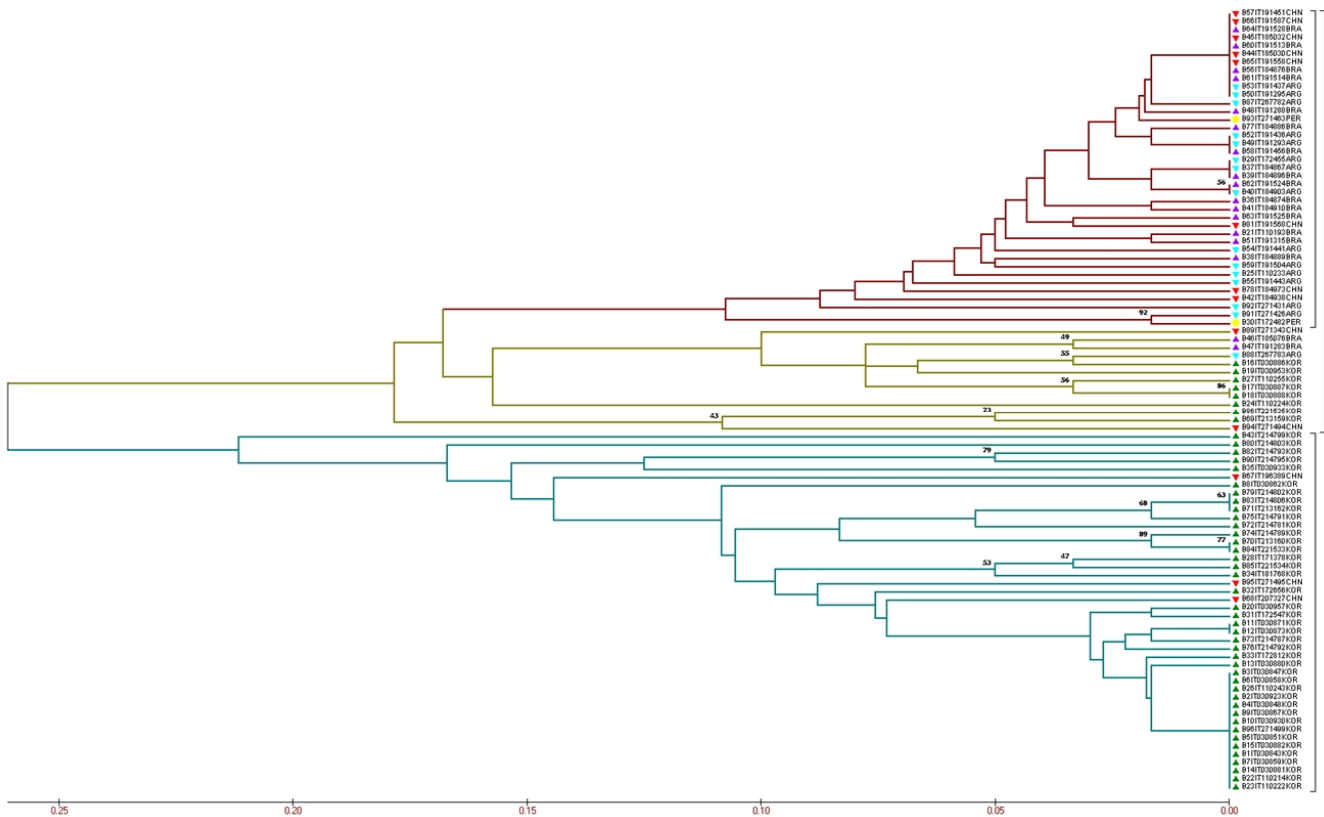


Fig. 1. Evaluation of evolutionary relationships among the 96 peanut accessions based on the 30 CAPS markers. The green mark indicates the genotypes from Korea; the blue mark indicates the genotypes from Argentina; the purple mark indicates the genotypes from Brazil; the red mark indicates the genotypes from China; and the yellow mark indicates the genotypes from Peru. The different colors of the branch represent different groups.

Table 2. Pairwise population F_{ST} values among five different origins.

Origins [†]	KOR	BRA	ARG	CHN
BRA	0.311	-		
ARG	0.264	0.034	-	
CHN	0.159	0.083	0.056	-
PER	0.246	0.156	0.094	0.115

[†]ARG, Argentina; BRA, Brazil; CHN, China; KOR, Korea; PER, Peru

Table 3. Pairwise population matrix of Nei's genetic distance among five different origins.

Origins [†]	KOR	BRA	ARG	CHN
BRA	0.437	-		
ARG	0.390	0.012	-	
CHN	0.224	0.047	0.036	-
PER	0.284	0.083	0.051	0.071

[†]ARG, Argentina; BRA, Brazil; CHN, China; KOR, Korea; PER, Peru

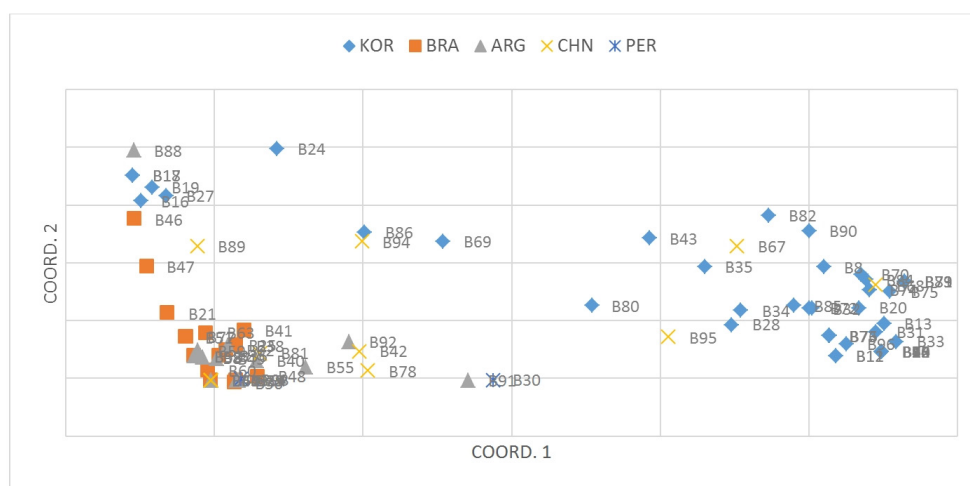


Fig. 2. Principal component analysis (PCoA) for the 96 peanut accessions based on the 30 CAPS markers; each point represents one accession and individuals are colored according to their origins. Coord.1 (49.86%) and Coord.2 (12.95%) refer to the first and second principal component, respectively. ARG is Argentina; BRA is Brazil; CHN is China; KOR is Korea; PER is Peru.

CHN, KOR and PER, PER and BRA had F_{ST} values ranged from 0.15 to 0.25 showed a high differentiation. Accessions from BRA and CHN, ARG and CHN, ARG and PER, PER and CHN had F_{ST} values ranged from 0.15 to 0.25 presented moderate differentiation. Accessions from BRA and ARG had F_{ST} values less than 0.05 indicating that the differentiation could be negligible.

The value of the Nei's genetic distance ranged from 0.012 to 0.437 (Table 3). The accessions from BRA and ARG presented the lowest genetic dissimilarity (0.012). The accessions from

KOR and BRA presented the highest genetic dissimilarities (0.437). The highest genetic dissimilarities were observed accessions from KOR between other four origins while genetic dissimilarities of accessions from other four origins had less than 0.1.

The pattern of PCoA (Fig. 2) was similar with the results of the UPGMA tree. The first two axes accounted for 65.03% of the total variation, and the 96 accessions were divided into three broad groups across the first two axes. The first axes separate the KOR accessions into two parts. However, Korea accessions

Table 4. Genetic diversity indices in five subpopulations.

Origin	N ^a	N _a ^b	N _e ^c	I ^d	H _O ^e	H _E ^f	uH _E ^g	F ^h	%P ⁱ
KOR	49	1.967	1.505	0.492	0.070	0.320	0.324	0.828	96.67%
BRA	17	1.500	1.214	0.206	0.078	0.130	0.134	0.552	50.00%
ARG	15	1.733	1.244	0.282	0.084	0.170	0.176	0.684	73.33%
CHN	13	1.867	1.467	0.430	0.077	0.282	0.293	0.813	86.67%
PER	2.	1.267	1.227	0.172	0.083	0.121	0.161	0.250	26.67%
Total	19	1.667	1.331	0.316	0.079	0.205	0.218	0.705	66.67%

^aNo. of Alleles; ^bNo. of Different Alleles; ^cNo. of Effective Alleles = 1 / (Sum pi²);

^dShannon's Information Index = -1 * Sum (pi * Ln (pi)); ^e Observed Heterozygosity = No. of Hets / N; ^f Expected Heterozygosity = 1 - Sum pi²; ^g Unbiased Expected Heterozygosity = (2N / (2N-1)) * H_e; ^h Fixation Index = (H_e - H_o) / H_e = 1 - (H_o / H_e); ⁱPercentage of Polymorphic Loci

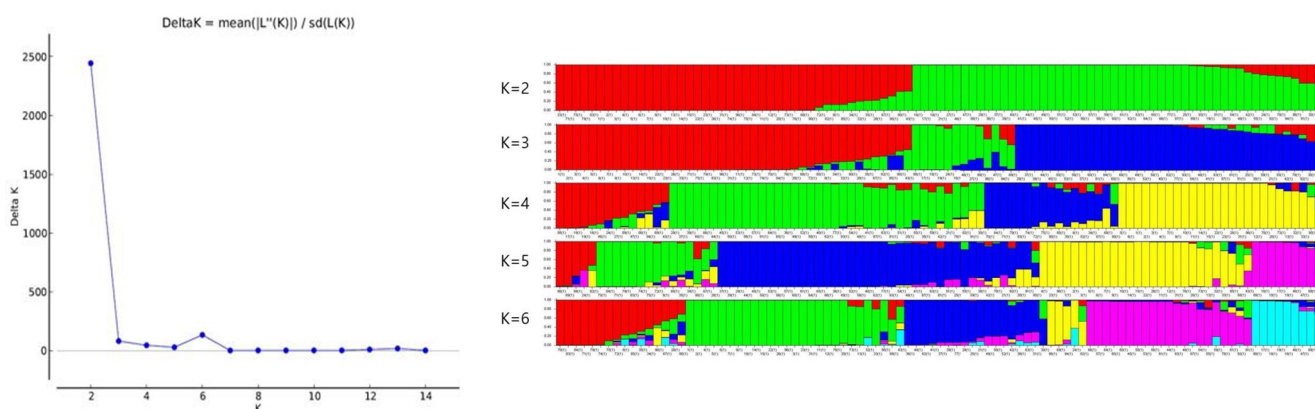


Fig. 3. (A) Estimation of the number of populations for K ranging from 2 to 15 by ΔK values, (B) Estimated genetic structure for K=2 to K=6.

formed a less sticky block with the others. The intermixing of color across the coordinates, further support the UPGMA tree with SNP marker that there is no location-specific grouping (Singh *et al.*, 2013) between accessions from BRA and ARG.

Genetic diversity indices including H_O (observed heterozygosity), H_E (expected heterozygosity), and the Fixation Index (F) were calculated (Table 4). For each group, the H_O and H_E calculated using all SNPs by observed genotype frequencies. The F values ranged from -1 to +1. Negative values indicate excess of heterozygosity. Values close to zero expected under random mating while a mass of positive values indicate inbreeding or undetected null alleles. For the 96 peanut individuals, mean H_O ranged from 0.070 (KOR) to 0.084 (ARG). The lowest mean H_E was found in the population from PER (0.121), whereas the highest was in the population from KOR (0.320). Across the origins, H_O (0.079) was significantly less than H_E (0.205).

Population structure

At $K = 2$, we found maximum Δk (Fig. 3A) values that were plotted against the K to confirm the number of populations. Another lower peak was shown at $K = 6$ (Fig. 3A). When most accessions divided into the two subpopulations ($K = 2$, Fig. 3B), a large portion of accessions from Korea belonged to one subgroup (red) while another subgroup (green) revealed features of accessions from other four origins. As we continue to divide subgroups carefully, there is a new division into the subgroups. The most divergent subgroups by origin were formed at $K = 6$, but all subgroups are mixed in origin. In the red, green, and yellow subpopulations, most accessions derived from KOR with only four accessions from CHN. However, the subpopulation labeled with dark blue were mainly from ARG and BRA, pink and light blue subpopulations mixed with different areas, which coincided with results of the UPGMA tree and PCoA.

DISCUSSION

Most crops including peanut have undergone a significant loss of genetic diversity during both the evolution and cultivation (Smykal *et al.*, 2018). The rich genetic diversity of genetic resources is a precondition for improvement in productivity and other goals in crop breeding programs. Genetic heterozygosity (H) known as genetic diversity could reflect the degree of genetic consistency of in the population (Tambasco-Talhari *et al.*, 2005). The lower H values represent that the higher genetic consistency and the lower genetic variation in the population. In the present study, the H_O was significantly lower than the H_E regardless the origins. This result might be directly affected by low heterozygosity of the tested peanut accessions or a general characteristic of peanut species with low genetic diversity. Besides, the low genetic diversity in the peanut accessions might be results of long-term peanut germplasm collection conducting with a lack of understanding of the genetic background or too much emphasis on the phenotypic variations.

The genetic diversity of the 96 peanut accessions was analyzed by cluster analysis. According to the SNP marker data, the 96 accessions were divided into two subpopulations. Most of the analyzed germplasms come from KOR divided with other germplasms. Genotypes from other origins were mixed in the same group, in this group, some peanut accessions from Korea formed a small separate group. Although low genetic diversity in peanut germplasm has been reported, analyses for population structure and clustering indicated that clear genetic differentiation between germplasm from KOR and other four countries. The STRUCTURE analysis reveals the existence of two subpopulations consistent with the clustering results based on genetic diversity. Our study identified the genetic differentiation in the peanut accessions from the five origins and this result could provide fundamental and visible information for enhancing genetic diversity studies and for finding novel traits in peanut breeding programs.

According to the geographical origin of different peanut accessions, the origins of peanut accessions are divided into East Asia and South America. The East Asia section includes peanut accessions from China and South Korea, while the South America section includes peanut accessions from Argentina, Brazil and Peru. In terms of geographical distribution, the peanut accessions from China and South Korea are the closest, while the

other accessions from Argentina, Brazil and Peru are the closest. The F_{st} values between ARG, BRA, CHN and PER were small, indicating that there was a small genetic differentiation between the four populations. However, the F_{st} value between KOR and ARG, BRA, CHN and PER is large, which indicates that the genetic distance between peanut varieties from Korea and those from ARG, BRA, CHN and PER is large, and there is a high genetic differentiation between populations, indicating that there is a significant difference between groups from Korea and other groups. Moreover, the F_{st} value between KOR and CHN was relatively small compared with that between KOR and ARG, BRA and PER, indicating that the genetic differentiation of peanut accessions in KOR and CHN was small. Geographically, it corresponds to the distribution characteristics of East Asia and South America. The long distance geographical separation of East Asia and South America is the reason for the high genetic diversity between KOR and South American peanut populations generally regarded as the origin of peanuts. According to the results of population structure, there are abundant genetic differences between peanut accessions from Korea and those from other sources, which may be the result of human selection, or the peanut accessions from Korea are excellent adapted to the local ecological environment. Also, it is necessary to introduce more peanut germplasm resources into Korean peanut accessions to expand the genetic diversity of Korean peanut accessions in future breeding programs.

In the latest research, peanuts made significant progress in the whole genome sequencing, providing a huge amount of molecular marker information and gene annotations, despite the complexity of genome of cultivated peanut (Bertioli *et al.*, 2016). Especially, SNP markers have good genetic affinity and can directly reflect a genetic diversity in the accessions at the DNA sequence level (Ren *et al.*, 2013). In this study, CAPS markers developed from SNPs clearly separated all accessions into the distinct subpopulations. Therefore, the use of molecular markers including CAPS might be a useful tool to determine genetic diversity and population structure in peanut.

In summary, this investigation about information on genetic diversity is helpful for developing appropriate scientific strategy for peanut breeding (Landjeva *et al.*, 2006) and it can be a great tool for genotype selection in a breeding program. Because of the large size of genome in the peanut species, it is necessary to use molecular markers, and breeders can use the molecular

marker data to select the required information in the absence of any pedigree information. The results obtained from this study showed that the successful application of SNP information derived from re-sequencing based on NGS technology, and this study also proved availability of the CAPS marker to figure out genetic diversity and population structure using 96 peanut accessions.

ACKNOWLEDGEMENTS

This work was supported by a 2-year Research Grant of Pusan National University.

REFERENCES

- Bertioli, D. J., S. B. Cannon, L. Froenicke, G. Huang, A. D. Farmer, E. K. Cannon, X. Liu, D. Gao, J. Clevenger, and S. Dash. 2016. The genome sequences of *Arachis duranensis* and *Arachis ipaensis*, the diploid ancestors of cultivated peanut. *Nature Genetics* 48(4) : 438-446.
- Bertioli, D. J., S. C. Leal-Bertioli, and H. T. Stalker. 2016. The peanut genome: The history of the consortium and the structure of the genome of cultivated peanut and its diploid ancestors. *Peanuts*, Elsevier: 147-161.
- Chen, X., Q. Lu, H. Liu, J. Zhang, Y. Hong, H. Lan, H. Li, J. Wang, H. Liu, and S. Li. 2019. Sequencing of cultivated peanut, *Arachis hypogaea*, yields insights into genome evolution and oil improvement. *Molecular Plant* 12(7) : 920-934.
- Dhillon, S. S., A. V. Rake, and J. P. Miksche. 1980. Reassociation kinetics and cytophotometric characterization of peanut (*Arachis hypogaea* L.) DNA. *Plant Physiology* 65(6) : 1121-1127.
- Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. *Molecular Ecology* 14(8) : 2611-2620.
- FAO. 2020. Food Outlook - Biannual Report on Global Food Markets. Rome, Italy.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39(4): 783-791.
- Feng, S., X. Wang, X. Zhang, P. M. Dang, C. C. Holbrook, A. K. Culbreath, Y. Wu, and B. Guo. 2012. Peanut (*Arachis hypogaea*) expressed sequence tag project: progress and application. *Comparative and Functional Genomics* 2012.
- Grasso, A. N., V. Goldberg, E. A. Navajas, W. Iriarte, D. Gimeno, I. Aguilar, J. F. Medrano, G. Rincón, and G. Ciappesoni. 2014. Genomic variation and population structure detected by single nucleotide polymorphism arrays in Corriedale, Merino and Creole sheep. *Genetics and Molecular Biology* 37(2) : 389-395.
- Kang, Y. J., Y.-K. Ahn, K.-T. Kim, and T.-H. Jun. 2016. Resequencing of *Capsicum annuum* parental lines (YCM334 and Taeon) for the genetic analysis of bacterial wilt resistance. *BMC Plant Biology* 16(1) : 235.
- Khera, P., H. Upadhyaya, M. Pandey, M. Roorkiwal, M. Sriswathi, P. Janila, Y. Guo, M. McKain, E. Nagy, and S. Knapp. 2013. SNP-based genetic diversity in the reference set of peanut (*Arachis* spp.) by developing and applying cost-effective KASPar genotyping assays. *Plant Genome* 6 : 1-11.
- Kim, K.-S., D. Lee, S. B. Bae, Y.-C. Kim, I.-S. Choi, S. T. Kim, T.-H. Lee, and T.-H. Jun. 2017. Development of SNP-Based Molecular Markers by Re-Sequencing Strategy in Peanut. *Plant Breeding and Biotechnology* 5(4) : 325-333.
- Kottapalli, K. R., M. D. Burow, G. Burow, J. Burke, and N. Puppala. 2007. Molecular characterization of the US peanut mini core collection using microsatellite markers. *Crop Science* 47(4) : 1718-1727.
- Landjeva, S., V. Korzun, and G. Ganeva. 2006. Evaluation of genetic diversity among Bulgarian winter wheat (*Triticum aestivum* L.) varieties during the period 1925–2003 using microsatellites. *Genetic Resources and Crop Evolution* 53(8) : 1605-1614.
- Lee, J., N. K. Izzah, M. Jayakodi, S. Perumal, H. J. Joh, H. J. Lee, S.-C. Lee, J. Y. Park, K.-W. Yang, and I.-S. Nou. 2015. Genome-wide SNP identification and QTL mapping for black rot resistance in cabbage. *BMC Plant Biology* 15(1) : 1-11.
- Peakall, R. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6(1) : 288-295.
- Rasheed, A., Y. Hao, X. Xia, A. Khan, Y. Xu, R. K. Varshney, and Z. He. 2017. Crop breeding chips and genotyping platforms: progress, challenges, and perspectives. *Molecular Plant* 10(8) : 1047-1064.
- Ren, J., D. Sun, L. Chen, F. M. You, J. Wang, Y. Peng, E. Nevo, D. Sun, M.-C. Luo, and J. Peng. 2013. Genetic diversity revealed by single nucleotide polymorphism markers in a worldwide germplasm collection of durum wheat. *International Journal of Molecular Sciences* 14(4) : 7061-7088.
- Ren, X.-P., H.-F. Jiang, B.-S. Liao, X.-J. Zhang, Y. Lei, G.-Q. Huang, L.-Y. Yan, and Y.-N. Chen. 2011. Distributing and genetic diversity of high oleic acid germplasm in Peanut (*Arachis hypogaea* L.) core collection of China. *Journal of Plant Genetic Resources* 12(4) : 513-518.
- Robledo, G., G. Lavia, and G. Seijo. 2009. Species relations among wild *Arachis species* with the A genome as revealed by FISH mapping of rDNA loci and heterochromatin detection. *Theoretical and Applied Genetics* 118(7) : 1295-1307.
- Saghai-Marooif, M. A., K. M. Soliman, R. A. Jorgensen and R. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences* 81(24) : 8014-8018.

- Seijo, G., G. I. Lavia, A. Fernández, A. Krapovickas, D. A. Ducasse, D. J. Bertioli, and E. A. Moscone. 2007. Genomic relationships between the cultivated peanut (*Arachis hypogaea*, Leguminosae) and its close relatives revealed by double GISH. *American Journal of Botany* 94(12) : 1963-1971.
- Singh, N., D. R. Choudhury, A. K. Singh, S. Kumar, K. Srinivasan, R. Tyagi, N. Singh, and R. Singh. 2013. Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of Indian rice varieties. *PLoS One* 8(12) : e84136.
- Smartt, J., W. Gregory, and M. P. Gregory. 1978. The genomes of *Arachis hypogaea*. L. Cytogenetic studies of putative genome donors. *Euphytica* 27(3) : 665-675.
- Smýkal, P., M. Nelson, J. Berger, and E. Von Wettberg. 2018. The Impact of Genetic Changes during Crop Domestication. *Agronomy* 8(7) : 119.
- Tambasco-Talhari, D., M. M. d. Alencar, C. C. P. d. Paz, G. M. d. Cruz, A. d. A. Rodrigues, I. U. Packer, L. L. Coutinho, and L. C. d. A. Regitano. 2005. Molecular marker heterozygosities and genetic distances as correlates of production traits in F1 bovine crosses. *Genetics and Molecular Biology* 28(2) : 218-224.
- Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24(8) : 1596-1599.
- Tamura, K., M. Nei, and S. Kumar. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* 101(30) : 11030-11035.
- Temsch, E. M. and J. Greilhuber. 2000. Genome size variation in *Arachis hypogaea* and *A. monticola* re-evaluated. *Genome* 43(3) : 449-451.
- Wang, Y., W. Liu, L. Xu, Y. Wang, Y. Chen, X. Luo, M. Tang, and L. Liu. 2017. Development of SNP markers based on transcriptome sequences and their application in germplasm identification in radish (*Raphanus sativus* L.). *Molecular Breeding* 37(3) : 26.
- Yang, H., Y. Tao, Z. Zheng, C. Li, M. W. Sweetingham, and J. G. Howieson. 2012. Application of next-generation sequencing for rapid marker development in molecular plant breeding: a case study on anthracnose disease resistance in *Lupinus angustifolius* L. *BMC Genomics* 13(1) : 318.
- Zhou, X., Y. Xia, X. Ren, Y. Chen, L. Huang, S. Huang, B. Liao, Y. Lei, L. Yan, and H. Jiang. 2014. Construction of a SNP-based genetic linkage map in cultivated peanut based on large scale marker development using next-generation double-digest restriction-site-associated DNA sequencing (ddRAD seq). *BMC Genomics* 15(1) : 351.