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Original Research Article

Selection of Resistant Varieties to *Aspergillus flavus* by Determination of Aflatoxin B_1 Content in Korean Peanut (*Arachis hypogaea* L.) Accessions

Seungah Han[1], Byeong-Cheol Kim[2], Jungmin Ha[3,†], and Tae-Hwan Jun[4,5,†]

ABSTRACT Peanuts, also known as groundnuts (*Arachis hypogaea* L.), are globally recognized as a vital oilseed crop. Peanuts are rich in proteins (e.g., arginine), oils (e.g., oleic acid and linoleic acid), fiber, vitamins (e.g., niacin and tocopherol), and carbohydrates and are consumed worldwide. However, the presence of aflatoxin (AF) has garnered substantial attention since its initial discovery as the causative agent of Tukey's X disease in the United Kingdom in 1960. Among the 18 aflatoxins identified, aflatoxin B_1 (AFB_1) has the highest toxic activity and causes hepatocellular carcinoma. It is classified as Group I by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO). The present study was conducted to evaluate aflatoxin B_1 resistance of 102 peanut accessions and select putative aflatoxin B_1-resistant peanut accessions to aflatoxin B_1. One hundred and one Korean germplasms harvested in 2020 were inoculated with *A. flavus* to identify aflatoxin-resistant cultivars, and the aflatoxin B_1 concentration was measured using an ultra-performance liquid chromatography-photodiode array detector. Twenty-six accessions with aflatoxin B_1 concentrations lower than those of the check plant 55-437 were chosen for the development of aflatoxin-resistant varieties in Korea. As Korean aflatoxin-resistant varieties have not yet been developed, the findings of the present study are expected to provide useful information for the development of aflatoxin-resistant cultivars.

Keywords : aflatoxin, peanut, Percent Seed Infection Index, resistant cultivar

Peanut, also known as groundnut (*Arachis hypogaea* L.), is one of the world's most important edible oil crops (Syed *et al*., 2021). Peanuts are consumed worldwide and are rich in proteins (e.g., arginine), oils (e.g., oleic acid and linoleic acid), fiber, vitamins (e.g., niacin and tocopherol), and carbohydrates (Andersen *et al*., 1998; Settaluri *et al*., 2012).

Mycotoxins are secondary metabolites produced by a variety of fungi such as *Aspergillus, Alternaria, Fusarium*, and *Penicillium*. The Food and Agriculture Organization (FAO) reported that 25% of food worldwide is contaminated with mycotoxins (Nazhand *et al*., 2020). Aflatoxin (AF) is the most toxic mycotoxin produced by the *Aspergillus* genus, which includes *Aspergillus flavus* (*A. flavus*) and produces aflatoxin B_1 (AFB_1) and B_2, and *Aspergillus parasiticus* (*A. parasiticus*), which produces aflatoxins B_1, B_2, G_1, and G_2 (Klich, 2007). Aflatoxin B_1 produced by *A. flavus* is the most widespread and toxic of the approximately eighteen types of aflatoxin (Yunus et *al*., 2011). *A. flavus*, which is more common in subtropical and tropical climates can grow at a wide range of temperatures (19–35°C), with incremental growth and aflatoxin production occurring at 28–30°C (Magan *et al*., 2011). *A. flavus* can survive for up to three years as sclerotia or conidia in soil and as mycelia in infected plant tissue (Wicklow *et al*., 1993). The sclerotium germinates into mycelium under favorable environmental conditions, producing conidiophores that are dispersed through the air and infect various crops (Horn, 2003; Klich, 2007).

[1)]Graduate Student, Department of Plant Bioscience, Pusan National University, Miryang 50463, Republic of Korea
[2)]Graduate Student, Department of Plant Science, Gangneung-Wonju National University, Gangneung 25457, Republic of Korea
[3)]Assistant Professor, Department of Plant Science, Gangneung-Wonju National University, Gangneung 25457, Republic of Korea
[4)]Professor, Department of Plant Bioscience, Pusan National University, Miryang 50463, Republic of Korea
[5)]Professor, Life and Industry Convergence Research Institute, Pusan National University, Miryang 50463, Republic of Korea

[†]Corresponding author: Jungmin Ha; (Phone) +82-33-640-2352; (E-mail) j.ha@gwnu.ac.kr
Tae-Hwan Jun; (Phone) +82-55-350-5507; (E-mail) thjun76@pusan.ac.kr
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Aflatoxin is destroyed between 237 and 306°C is difficult to totally degrade even with chemical treatment, making it difficult to decrease aflatoxin levels in general practice (Pandey *et al*., 2019). Many crops, including cereals (e.g., maize, sorghum, wheat, and rice), oilseeds (e.g., soybeans, peanuts, and cottonseed), spices (e.g., chilies, peppers, and ginger), and nuts (e.g., pistachios and almonds), have been damaged by aflatoxins produced by *A. flavus* (Blount, 1961; Stössel, 1986; Jaime-Garcia *et al*., 2003; Giray *et al*., 2007; Neme & Mohammed, 2017). The toxin is taken into the human body directly or indirectly by ingesting contaminated foods or the by-products of animals that have consumed toxin-contaminated feed (Ostry *et al*., 2017). The excessive intake of aflatoxin can induce acute poisoning (aflatoxicosis), which is frequently fatal due to liver damage (Pitt, 2000). Aflatoxins are also genotoxic (Verma, 2004) and can induce DNA damage, which causes cancer in animals including humans (Wu, 2015). The International Agency for Research on Cancer (IARC) classified aflatoxin B_1 as carcinogenic (Group 1) for hepatocellular carcinoma (WHO and IARC, 1993). The production of aflatoxin M_1 in milk by animals fed aflatoxin B_1-contaminated feed is an example of a secondary impact (Guan *et al*., 2011).

Aflatoxin (AF) resistance in peanuts has been studied since its discovery in 1960, and researchers have reported three mechanisms of host-pathogen resistance, including in vitro seed colonization (IVSC), pre-harvest aflatoxin contamination (PAC), and aflatoxin production (AP) in diverse genetic backgrounds (Pandey *et al*., 2019; Pickova *et al*., 2021). Among these mechanisms, resistant varieties 55-437, J11, PI337394F, and AR-1 were chosen for the IVSC mechanism (Commey *et al*., 2021; Nayak *et al*., 2017; Wilson *et al*., 1977; Rao *et al*., 1989). F1334 and F1344 were chosen as resistant cultivars for the PAC mechanism (Holbrook *et al*., 2000), and U 4-7-5 and VRR 245 were chosen for the AP mechanism (Mehan *et al*., 1986).

Detection techniques for aflatoxin can be categorized as chromatographic, immunochemical, and spectroscopic. High-performance liquid chromatography (HPLC) has been reported as the best technique for quantifying aflatoxins in foods because of its high sensitivity (Wacoo *et al*., 2014). The present study was conducted to evaluate the aflatoxin B_1 resistance of 102 peanut accessions using ultra-performance liquid chromatography (UPLC) and select putative aflatoxin B_1-resistant peanut accessions.

MATERIALS AND METHODS

Plant material

A total of 102 peanut accessions were used in this experiment (Supplementary Table S1). Among them, 101 peanut germplasms were obtained from the Korean National Agrobiodiversity Center, Rural Development Administration (RDA) Genebank Information Center (Wanju-gun, South Korea), including landraces, breeding lines, and cultivars. In addition, one aflatoxin-resistant peanut germplasm '55-437' was employed as a check plant (Nigam *et al*., 2009). The peanut accessions were planted and harvested in the experimental field of Pusan National University (Miryang, South Korea) in 2020. Each accession was planted in a two-row plot 1 m long with 0.2 m row spacing. Each plot was spaced 0.4 m apart in the planting route, with 0.9 m inter-row spacing to decrease cross-contamination. The seeds were planted at a density of two seeds/hole at a depth of 2 cm.

Inoculation with *Aspergillus flavus* strains

A. flavus strain KACC 45068, producing aflatoxin B_1 was obtained from the Korea Agricultural Culture Collection (Jeonju, South Korea). *A. flavus* conidia were cultured on potato dextrose agar (PDA) medium in a 90 mm petri dish at 29 ±1°C for 14 days. Conidia were collected and suspended in sterile water containing 0.05% Tween-20 (v/v), and the concentration of conidia in the suspension was calculated using a hemocytometer (DHC-N01, NanoEnTek, Seoul, Korea). An *A. flavus* conidial suspension of 2×10^6 CFU/mL was made in 0.05% Tween-20 solution. Ten healthy peanut seeds were selected for each accession harvested in 2020, sterilized with 75% ethanol for 1 min, and washed three times with sterile water for 13 min (Yu *et al*., 2019). Each seed was inoculated with 100 μL of conidia suspension. Peanuts inoculated with *A. flavus* were grown for seven days at 29 ± 1°C and 85% humidity in the dark.

Percent seed infection index estimation

The percent seed infection index (PSII) of each peanut seed inoculated for seven days was estimated (Fig. 1). The external infection of each seed was determined by visual inspection at four levels by the slight modification of a previously reported method (Yu *et al*., 2019). The fungal infection rating for the individual kernels of each germplasm and scale value was determined, and the percentage of severe external infections was calculated using the following equation:

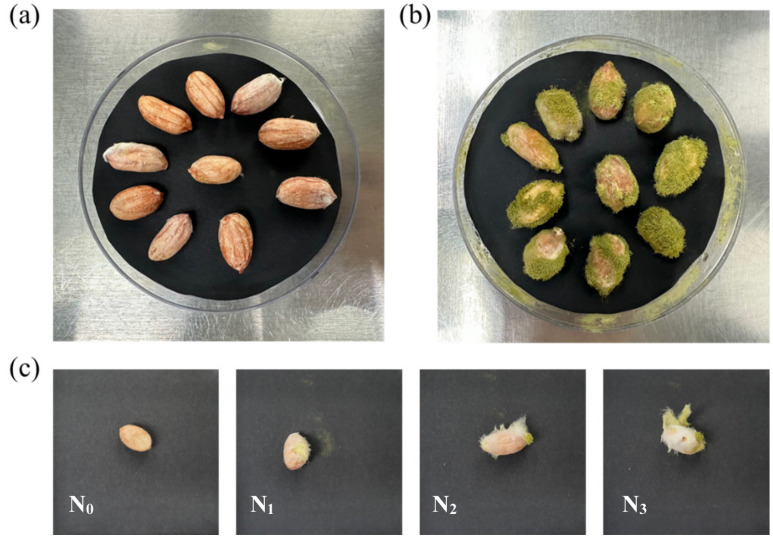

Fig. 1. Visual appearance of peanuts seven days after inoculation with *A. flavus*. (a) GWP 1, representing the resistant group with a 0% PSII value, (b) K-Ol, representing the susceptible group with a 100% PSII value, and (c) GWP 51, with a 73.3% PSII value. The grades from left to right are N_0 when the conidia surface was 0, N_1 from 0 to 1/4, N_2 from 1/4 to 1/2, and N_3 from 1/2 until the surface was fully formed.

$$PSII\,(\%) = \frac{N1 + N2 \times 2 + N3 \times 3}{N \times 3} \times 100$$

(where N is the total number of seeds and the level of spore coverage on the seed surface is represented by N_0 for seed surface infection: N_1 for 0 to 1/4 coverage, N_2 for 1/4 to 1/2 coverage, and N_3 for 1/2 to complete coverage.)

Quantitative analysis of aflatoxin B_1

Aflatoxin B_1 content in peanuts was quantified by referring to the Korean Food Code (2022) and the Association of Official Analytical Chemists (AOAC) Method 991.31 (AOAC, 1994). After co-culturing with *A. flavus* for seven days, all peanut seeds were rinsed with 75 % ethanol, dried at 110°C for two hours, and ground at full speed for 2 min using a Waring blender (Vicam, Watertown. MA, USA). Extract solution (40 mL of 70% MeOH with 1% NaCl (v/v)) was added to the ground sample and homogenized for 1 min with the blender and then filtered through Whatman No. 4 filter paper (Whatman, Buckinghamshire, UK). The purified extract of (10 mL) was added to 30 mL of 1% Tween-20 (v/v), filtered through glass fiber filter paper (GF/A, Whatman), and injected into an immunoaffinity column (AflaTest WB, Vicam, MA, USA). Then 20 mL of extract was injected into the immunoaffinity column and passed through at a rate of about one drop per second. Water (15 mL; Burdick & Jackson, Muskegon, MI, USA) was injected into the immunoaffinity column and passed it through at a rate of about one or two drops per second for washing. Elution was performed with 3 mL acetonitrile (Burdick & Jackson, Muskegon, MI, USA). The eluate was dried with nitrogen at 50°C. For derivatization, 0.2 mL of trifluoroacetic acid (TFA; Sigma-Aldrich, St. Louis, MO, USA) was added and left in the dark for 15 min, and 0.8 mL of 20% acetonitrile (v/v) was added and filtered with a 0.2 μm syringe filter. TFA derivatization, which is A chemical derivatization procedure, was conducted for UPLC analysis after pretreatment of the outer surface.

The concentration of aflatoxin B_1 was determined by UPLC (Shimadzu UPLC, Nexera System, Shimadzu, Kyoto, Japan) with a 250 mm × 4.6 mm, 5 μm C18 column (Agilent, Santa Clara, CA, USA). The system column heater maintained a column temperature of 40°C. A photodiode array detector (PDA; Shimadzu) was utilized as the UPLC detector. The aflatoxin standard used in the analysis was acquired from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, United States). Aflatoxin standard was also dried at 50°C with nitrogen, 0.2 mL of TFA was added and left in the dark for 15 min, and 0.8 mL of 20% acetonitrile (v/v) was added and filtered through a 0.2 μm syringe filter for derivatization. The mobile phase consisted of a mixture of water and acetonitrile (77:23, v/v), with a flow rate of 1 mL/min. The injection volume was 40 μL, and the injection

time was 20 min. Aflatoxin concentration was measured as the peak area using a regression equation. The aflatoxin B_1 content was determined using the formula specified in the Korean Food Code (2022).

$$y = \frac{x + 22542.9}{141909}$$

(where y is the concentration of aflatoxin B_1, and x is the peak area of aflatoxin B_1)

Resistance variety clustering method

The K-means clustering algorithm in R software was used to select accessions resistant to aflatoxin B_1 (Bock, 2008). Since the aflatoxin B_1 and PSII units diverged, standardization was performed to bring them into alignment (Mohamad & Usman, 2013). A within sum of squares (WSS) plot was used to explain the homogeneity within a cluster by determining the K value (number of clusters) (Makles, 2012). Visualization of the cluster results was performed using the Factoextra package in R software (Kassambara & Mundt, 2017).

RESULTS AND DISCUSSION

A total of 102 accessions including 101 Korean germplasms and 55-437, were inoculated with *A. flavus*. Seven days after inoculation, PSII was performed using 102 accessions, and all accessions were divided into four classes according to the degree of infection (Fig. 1).

After inoculation, UPLC-PDA analysis of aflatoxin B_1 was performed. The standard contained aflatoxin B_1, B_2, G_1, and G_2, and the TFA derivative detected aflatoxin G_1, B_1, G_2, and B_2 in that order. *A. flavus*, a fungus that produces aflatoxin B_1 and B_2, was also utilized in this experiment, and aflatoxin B_1 was primarily detected in sample analysis (Fig. 2).

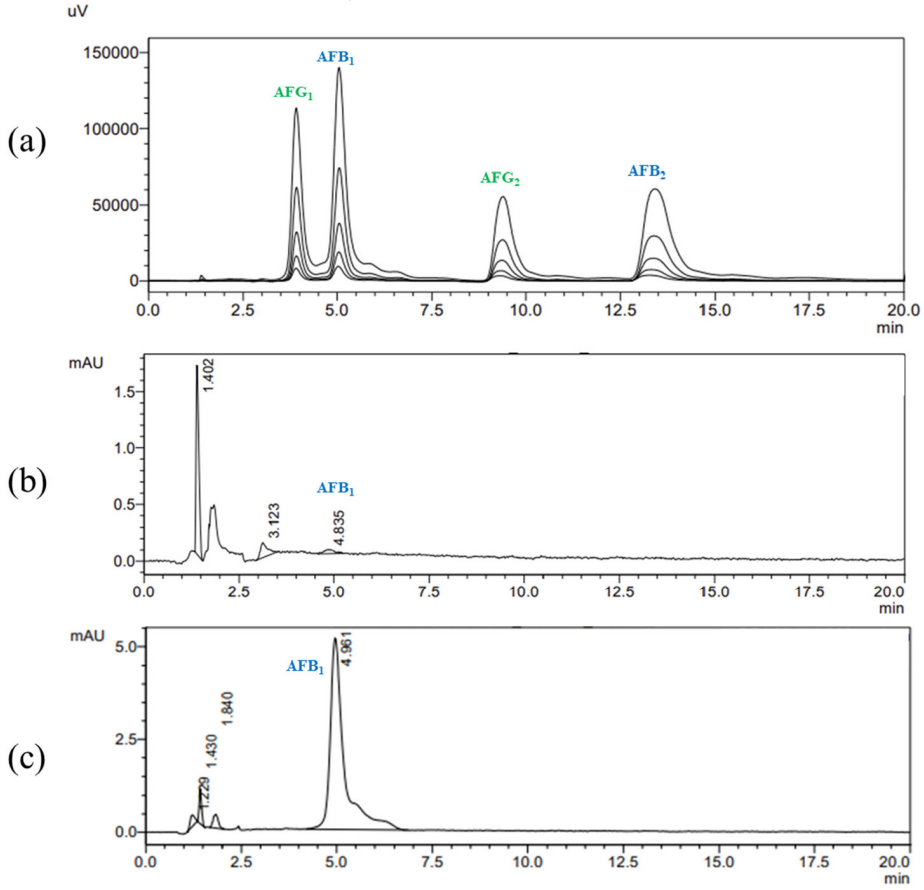

Fig. 2. Analysis of aflatoxin B_1 using UPLC-PDA in 101 Korean peanuts infected with *A. flavus*. (a) UPLC analysis of standards containing aflatoxin G_1, B_1, G_2, and B_2; (b) UPLC analysis of GWP 1 (0.599 μg/g), a resistant variety; and (c) K-Ol (2.150 μg/g), a susceptible variety.

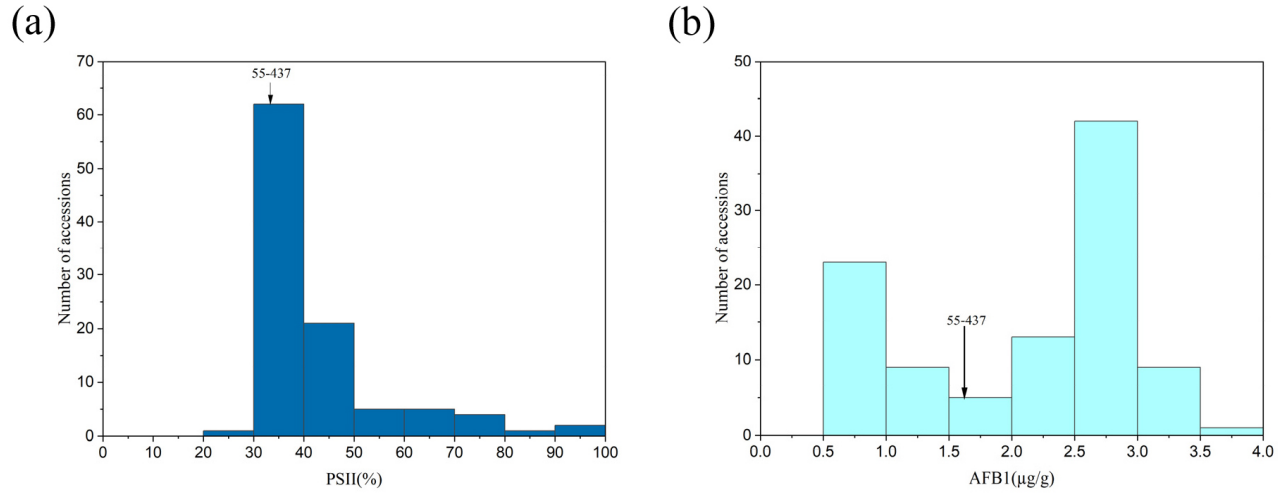

Fig. 3. Assessment of aflatoxin B_1 contamination in 101 peanut accessions from Korea. (a) Percent seed infection index (PSII) values for Korean peanut accessions, and (b) Aflatoxin B_1 levels in peanut accessions harvested in the 2020 season. Cultivar 55-437 serves as an aflatoxin-resistant cultivar that was used as the check plant.

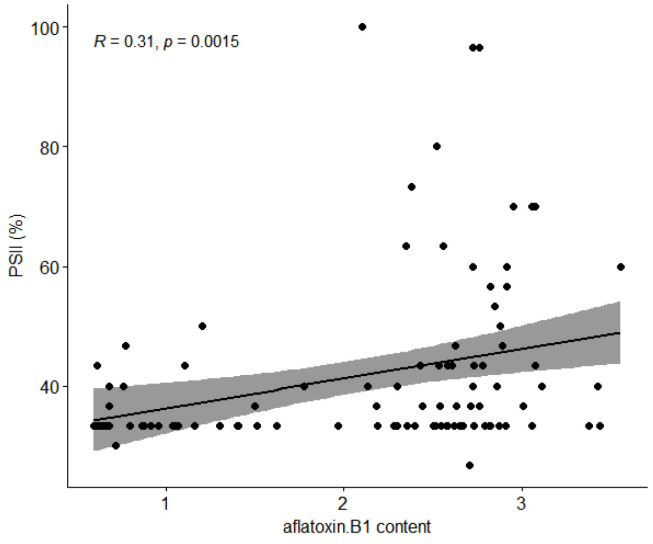

Fig. 4. Correlation analysis between aflatoxin B_1 content and Percent Seed Infection Index (PSII) values in 101 Korean germplasms infected with *A. flavus*. Pearson's analysis yielded a correlation coefficient (R) of 0.31, indicating a low correlation.

LC (liquid chromatography) is one of the most common chromatographic methods used to measure aflatoxin concentrations (Wacoo *et al*., 2014). However, unlike Enzyme-linked immunosorbent assay (ELISA) or Thin-layer chromatography (TLC), aflatoxin B_1, B_2, G_1, and G_2 fluorescence cannot be distinguished without a derivatization process. Existing techniques include pre-column derivatization with TFA and post-column derivatization with a photochemical reactor for enhanced detection (PHRED) and a KOBRA electrochemical cell system (Kok, 1994). In the case of aflatoxin B_1, the double bond in the dihydrofuran group is rapidly rehydrated, resulting in the formation of the fluorescent species B_{2a} via TFA derivatization, which is an analyzable form. In contrast to post-column derivatization, pre-column derivatization modifies the detection order from aflatoxin G_2-G_1-B_2-B_1 to aflatoxin G_1-B_1-G_2-B_2 (Woo *et al*., 2022).

Following culture, PSII values by visual inspection ranged from 26.6 to 100% (Fig. 3a). In this experiment, the fungus *A. flavus*, which produces aflatoxins B_1 and B_2, was used, and only aflatoxin B_1 content was evaluated. Aflatoxin B_1 content in peanut accessions harvested in 2020 ranged from 0.599 to 3.554 μg/g (Fig. 3b). The reported resistant accession 55-437 had a PSII value of 33.3% and aflatoxin B_1 levels of 1.623 μg/g. Pearson's correlation analysis revealed a correlation coefficient of 0.31 between the two values (Fig. 4).

Aflatoxin B_1 content and PSII values were compared to select peanut accession varieties resistant to aflatoxin B_1 in Korean varieties (Fig. 3). The K-means clustering algorithm in R software was utilized, and a value 4 was selected for K as a Within Sum of Squares (WSS) plot, resulting in four clusters (Fig. 5). The resistant cultivar 55-437 was assigned to cluster 2, which was chosen as the resistant group. The results showed that 26 accessions with both values less than those of 55-437 could be used to develop peanut varieties resistant to aflatoxin B_1 (Table 1).

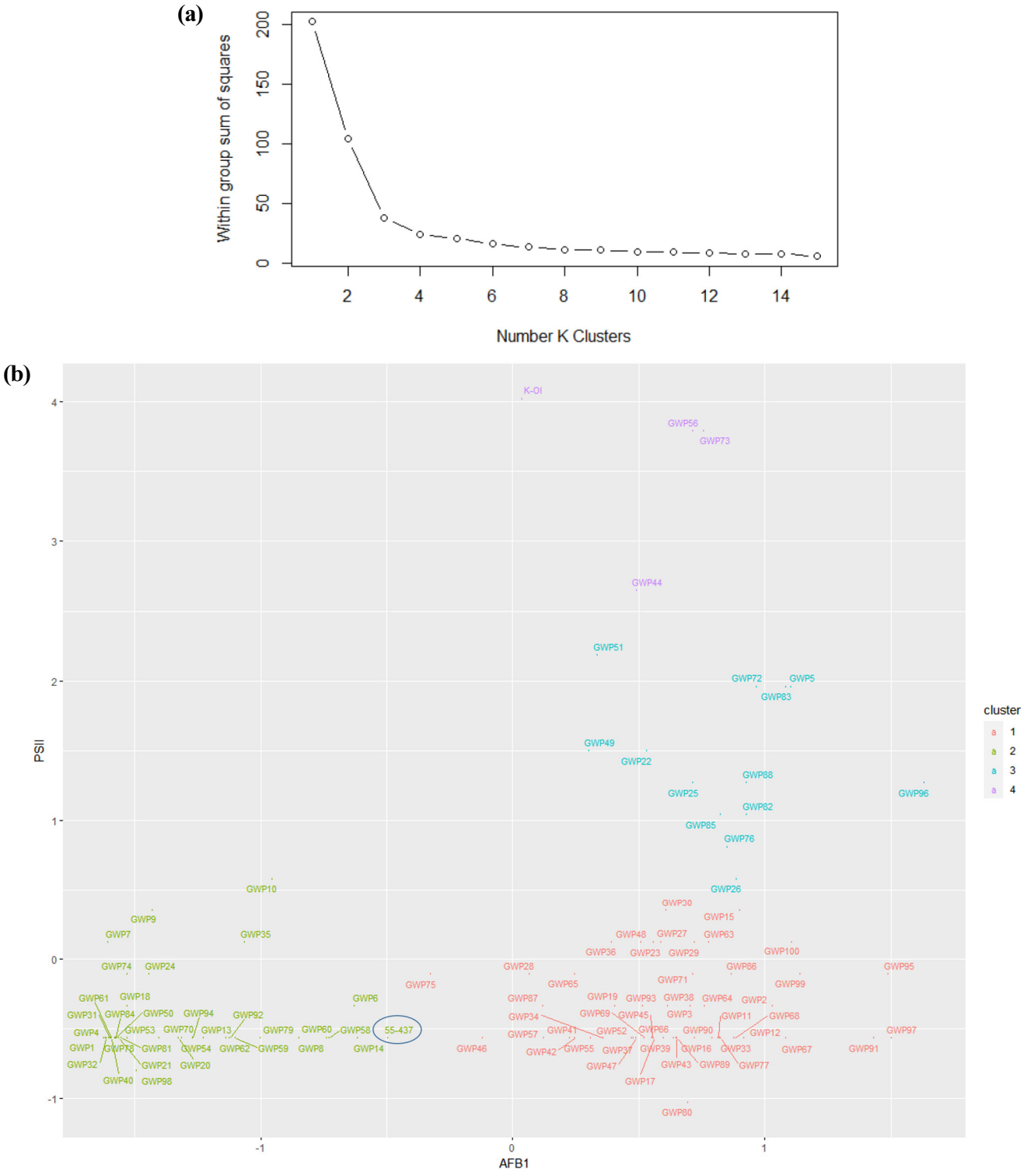

Fig. 5. Selection of aflatoxin B_1-resistant cultivars utilizing the K-means algorithm implemented in R to select resistant cultivars. (a) The Within-Cluster Sum of Squares (WSS) plot resulted in the formation of four distinct clusters. (b) The clustering plot illustrates the distribution of 101 Korean peanut accessions alongside cultivar 55-437 (check plant), based on their aflatoxin B_1 and PSII values, into four groups. Among these, Cluster 2 (light green) contains the check plant 55-437 (circled) and has been identified as the resistant group. The subsequent selection of 26 genetic resources from cluster 2 will be used to propagate cultivars resistant to aflatoxin B_1 in Korea.

Table 1. List of aflatoxin B_1-resistant peanut germplasms chosen from 101 Korean peanut accessions.

No.	Aflatoxin B_1 (μg/g)	PSII (%)	ID No.	Accession Name	Improvement status	Origin
GWP 1	0.599	33.33	IT 030842	Chungbuk Chungju-30842	Landrace	Korea, South
GWP 4	0.61	33.33	IT 030854	Chungnam Asan-30854	Landrace	Korea, South
GWP 8	1.304	33.33	IT 030866	Jeonnam Muan-30866	Landrace	Korea, South
GWP 13	0.959	33.33	IT 030930	Cheongsong 3	Breeding line	Korea, South
GWP 14	1.515	33.33	IT 030953	Gyeonggi Ganghwa-30953	Landrace	Korea, South
GWP 20	0.869	33.33	IT 104889	Gyeongnam Goseong-4889	Landrace	Korea, South
GWP 21	0.654	33.33	IT 108816	Gyeongbuk Wolseang-8816	Landrace	Korea, South
GWP 31	0.619	33.33	IT 110244	Jeonbuk Sunchang-10244	Landrace	Korea, South
GWP 32	0.606	33.33	IT 110245	Gyeonggi Gapyeong-10245	Landrace	Korea, South
GWP 40	0.626	33.33	IT 172541	Suweon 64	Breeding line	Korea, South
GWP 50	0.641	33.33	IT 172658	Suweon 34	Breeding line	Korea, South
GWP 53	0.684	33.33	IT 175811	Gyeongbuk Andong-2653	Landrace	Korea, South
GWP 54	0.879	33.33	IT 181763	Suwon 85	Breeding line	Korea, South
GWP 58	1.41	33.33	IT 191603	Suweon 100	Breeding line	Korea, South
GWP 59	1.073	33.33	IT 191604	Shinkwang Ttangkong	Cultivar	Korea, South
GWP 60	1.402	33.33	IT 191606	Suweon 103	Breeding line	Korea, South
GWP 61	0.625	33.33	IT 191608	Suweon 105	Breeding line	Korea, South
GWP 62	1.039	33.33	IT 191609	Suweon 106	Breeding line	Korea, South
GWP 70	0.798	33.33	IT 209224	Incheon Ganghwa-983389	Landrace	Korea, South
GWP 78	0.644	33.33	IT 213163	Iksan 17	Breeding line	Korea, South
GWP 79	1.164	33.33	IT 214792	Suweon 88	Cultivar	Korea, South
GWP 81	0.662	33.33	IT 214799	Iksan 7	Cultivar	Korea, South
GWP 84	0.64	33.33	IT 220411	Chungnsm Yeongi-17	Landrace	Korea, South
GWP 92	1.053	33.33	IT 310161	Ami	Cultivar	Korea, South
GWP 94	0.919	33.33	-	Pungsan	Cultivar	Korea, South
GWP 98	0.718	30	IT345355	Sangan	Cultivar	Korea, South

The objective of the study was to assess aflatoxin B_1 resistance and select resistant varieties from 101 Korean genetic resources. Clustering was used as the evaluation method, and a visual inspection was used to determine the PSII value. Aflatoxin B_1 content was quantitatively analyzed by UPLC-PDA, with reference to previously published papers, and based on that of 55-437 (check plant) (Waliyar *et al*., 2016; Yu *et al*., 2020). The correlation coefficient between the two values was as low as 0.31, indicating that their relationship was unreliable based on previous studies (Yu *et al*., 2019). Nonetheless, resistance to fungi should be considered to be due to two host-pathogen resistance types because they play an essential role in aflatoxin production in peanuts. Twenty-six genetic resources with lower values than the check plant were chosen as aflatoxin B_1-resistant cultivars. Since varieties resistant to aflatoxin B_1 have not yet been developed in Korea, the accessions will be used in the future to develop aflatoxin B_1-resistant cultivars.

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Supplementary Table 1. The information of 101 peanut accessions.

No.	ID No.	Accession Name	Improvement status	Origin	Region	Institution	Taxonomy
GWP1	IT 030842	Chungbuk Chungju-30842	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP2	IT 030844	Gyeongnam Jinju-30844	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP3	IT 030848	Gyeongnam Sanchung-30848	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP4	IT 030854	Chungnam Asan-30854	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP5	IT 185682	Chungbuk Okcheon-3343	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP6	IT 030862	Jeju Namkje-30862	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP7	IT 030863	Jeju Bukje-30863	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP8	IT 030866	Jeonnam Muan-30866	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP9	IT 030880	Kangwon Yangyang-30880	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP10	IT 030882	Kangwon Chunsung-30882	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP11	IT 030887	Chungnam Yesan-30887	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP12	IT 030923	Uiseong 4	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP13	IT 030930	Cheongsong 3	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP14	IT 030953	Gyeonggi Ganghwa-30953	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP15	IT 030957	Suweon 2	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP16	IT 030958	Suweon 4	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP17	IT 030960	Suweon 6	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP18	IT 030961	Suwon 8	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP19	IT 030962	Suwon 9	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP20	IT 104889	Gyeongnam Goseong-4889	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP21	IT 108816	Gyeongbuk Wolseang-8816	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP22	IT 110209	Suweon 20	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP23	IT 110219	Suweon 37	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP24	IT 110224	Ol Ttangkong	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP25	IT 110235	Suweon 47	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP26	IT 110237	Suweon 49	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*

Supplementary Table 1. The information of 101 peanut accessions (Continued).

No.	ID No.	Accession Name	Improvement status	Origin	Region	Institution	Taxonomy
GWP27	IT 110240	Suweon 42	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP28	IT 110241	Gyeonggi Hwaseong-10241	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP29	IT 110242	Gyeonggi Yeoju-10242	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP30	IT 110243	Kangwon Jungsun-10243	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP31	IT 110244	Jeonbuk Sunchang-10244	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP32	IT 110245	Gyeonggi Gapyeong-10245	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP33	IT 110246	Gyeongbuk Andong-10246	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP34	IT 121450	Gyeongbuk Sangju-7709	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP35	IT 144016	Saedeul Ttangkong	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP36	IT 155176	Gyeongbuk Sangju-5181	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP37	IT 172431	Suwon 83	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP38	IT 172432	Suwon 89	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP39	IT 172540	Suweon 62	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP40	IT 172541	Suweon 64	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP41	IT 172543	Suweon 66	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP42	IT 172544	Suweon 67	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP43	IT 172547	Suweon 70	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP44	IT 172549	Suweon 73	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP45	IT 172552	Suweon 35	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP46	IT 172554	Suweon 41	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP47	IT 172556	Nampung Ttang kong	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP48	IT 172655	Josaengjong	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP49	IT 172657	Suweon 32	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP50	IT 172658	Suweon 34	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP51	IT 172809	Jinpung	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP52	IT 172823	Iri 1	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*

Supplementary Table 1. The information of 101 peanut accessions (Continued).

No.	ID No.	Accession Name	Improvement status	Origin	Region	Institution	Taxonomy
GWP53	IT 175811	Gyeongbuk Andong-2653	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP54	IT 181763	Suwon 85	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP55	IT 185464	Iri 2	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP56	IT 185678	Jeonnam Jangseong-3339	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP57	IT 191602	Suwon 98	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP58	IT 191603	Suweon 100	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP59	IT 191604	Shinkwang Ttangkong	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP60	IT 191606	Suweon 103	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP61	IT 191608	Suweon 105	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP62	IT 191609	Suweon 106	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP63	IT 191610	Iri 4	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP64	IT 194509	Jeonnam Damyang-6277	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP65	IT 196354	Suweon 107	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP66	IT 196358	Milyang 5	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP67	IT 196359	Mil 16	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP68	IT 203652	Milyang 7	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP69	IT 207978	Chungnam Geumsan-17	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP70	IT 209224	Incheon Ganghwa-983389	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP71	IT 212142	Jeonnam Wando-115	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP72	IT 212144	Hogwang	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP73	IT 213157	Milyang 14	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP74	IT 213158	Milyang 15	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP75	IT 213159	Jagwang Ttangkong	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP76	IT 213160	Baekjung	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP77	IT 213162	Akwang Ttangkong	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP78	IT 213163	Iksan 17	Breeding line	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*

Supplementary Table 1. The information of 101 peanut accessions (Continued).

No.	ID No.	Accession Name	Improvement status	Origin	Region	Institution	Taxonomy
GWP79	IT 214792	Suweon 88	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP80	IT 214798	Daecheong Ttangkong	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP81	IT 214799	Iksan 7	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP82	IT 214800	Joan	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP83	IT 214804	Dagwang	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP84	IT 220411	Chungnsm Yeongi-17	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP85	IT 221532	Sangpyeong	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP86	IT 221533	Baekan	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP87	IT 221535	Charmwon	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP88	IT 267784	Kangwon Gosung-39	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP89	IT 271498	Kangwon Gosung-45	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP90	IT 271499	Gyeongnam Sanchung-73	Landrace	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP91	IT 304334	Milyang 59	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP92	IT 310161	Ami	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP93	-	Daegwang	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP94	-	Pungsan	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP95	-	Seonan	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP96	-	Ilpyeong	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP97	-	Yeonpung	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP98	-	Sangan	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP99	-	Jaseon	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
GWP100	-	Daan	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*
K-Ol	IT 310159	K-Ol	Cultivar	Korea, South	East Asia	Peanut. Germplasm. KOR	*Arachis hypogaea*