RESEARCH ARTICLE

Analysis of Genetic Diversity and Population Structure of Wild Strains and Cultivars Using Genomic SSR Markers in *Lentinula edodes*

Hwa-Yong Lee[a,b], Suyun Moon[b], Hyeon-Su Ro[c] (iD), Jong-Wook Chung[d] and Hojin Ryu[b]

[a]Department of Forest Science, Chungbuk National University, Cheongju, Republic of Korea; [b]Department of Biology, Chungbuk National University, Cheongju, Republic of Korea; [c]Division of Applied Life Science and Research Institute of Life Science, Gyeongsang National University, Jinju, Republic of Korea; [d]Department of Industrial Plant Science and Technology, Chungbuk National University, Cheongju, Republic of Korea

ABSTRACT

In this study, the genetic diversity and the population structure of 77 wild strains and 23 cultivars of *Lentinula edodes* from Korea were analyzed using 20 genomic SSRs, and their genetic relationship was investigated. The tested strains of *L. edodes* were divided into three sub-groups consisting of only wild strains, mainly wild strains and several cultivars, and mainly cultivars and several wild strains by distance-based analysis. Using model-based analysis, *L. edodes* strains were divided into two subpopulations; the first one consisting of only wild strains and the second one with mainly cultivars and several wild strains. Moreover, AMOVA analysis revealed that the genetic variation in the cultivars was higher than that in the wild strains. The expected and observed heterozygosity and values indicating the polymorphic information content of *L. edodes* cultivars from Korea were also higher than that of the wild strains. Based on these results, we presume that the cultivars in Korea have developed by using numerous strains from other countries. In conclusion, the usage of wild strains for the development of new cultivars could improve the adaptability of *L. edodes* to biotic and abiotic stress.

1. Introduction

Lentinula edodes is a white rot fungus that is found on dead or rotten tree stumps in forests and it is geographically distributed all over Asia, Australia, and the Americas [1]. This is an edible mushroom, which is not only a highly valuable food source but also contains important pharmacological components such as lentinan [2,3]. The agricultural cultivation of *L. edodes* was first reported in China about 800 years ago [4] and modern cultivation methods using the pure cultivated mycelium were developed in Japan in the 1930s [5]. *Lentinula edodes* contributes to approximately 22% of the total mushroom production in the world [6] and the main cultivation region is East Asia including Korea, China, and Japan [7]. This mushroom has been recently highlighted in Europe, North America, and Africa due to its nutritional and pharmacological importance [8].

The development of novel varieties can be achieved by selecting and introducing new resources from crops [9]. In particular, the introduction of wild resources and core collection has been regarded as an effective way to improve the breeding for the development of new cultivars with improved adaptability to biotic and abiotic stress [10,11]. Securing the genetic potential of wild genetic resources is a major tool for crop improvement [12]. Genetic diversity and population structure analysis can provide important information regarding the origin and the evolution of the species, which can aid in selecting potential genetic resources for future use [13]. The genetic diversity can be determined by using morphological characteristics as well as DNA marker analysis [14]. However, the usage of morphological characteristics has been reported to have certain disadvantages as the expression of the phenotypes is influenced by the growth stages and various environmental factors [15]. DNA markers are comparatively less affected by various environmental factors and growth stages [16]. Moreover, analysis using DNA markers is simple and rapid, reproducible by experiment regardless of environmental conditions, and aspects such as the accurate monitoring of seed purity, determination of cultivars, can be performed at low cost [17,18]. Analysis with DNA markers include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length

polymorphism (AFLP), inter-simple sequence repeats (ISSRs), sequence characterized regions (SCARs), sequence tag sites (STSs), cleaved amplified polymorphic sequences (CAPS), microsatellites or simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs), and these methods have been extensively used for current breeding programs [19] and genetic diversity analysis [20]. SSRs, which are randomly repeated DNA sequences, are widely distributed throughout the genome with 1–6 base pairs per unit length, and are codominant, highly polymorphic, and highly reproducible [21]. Hence, this marker is ideal for the analysis of genetic diversity and population structure [22].

In this study, to investigate the genetic relationship between the wild and cultivated strains of *L. edodes* in Korea, the genetic diversity and population structure analyses of 77 wild strains and 23 cultivars were performed using 20 genomic SSRs.

2. Materials and methods

2.1. Fungal material

A total of 100 strains of *L. edodes* consisting of two types of wild strains and cultivars were included in this study. Seventy-seven wild strains were collected and maintained at the National Institute of Forestry Science, Forest Mushroom Research Center and Chungbuk National University, and 23 cultivars were developed by the National Institute of Forestry Science and Forest Mushroom Research Center (Figure 1, Supplementary Table 1).

2.2. DNA extraction and SSR analysis

The strains were incubated in liquid potato dextrose broth (PDB) media at 25 °C for approximately 10 days in darkness. The cultured mycelia were finely ground using liquid nitrogen and the DNA was extracted. DNA extraction was performed using a GenEX Plant Kit (Geneall, Seoul, Korea) and the extracted DNA was quantified to be at 20 ng/μL using a K5600 micro spectrophotometer.

The 20 SSR markers used in this study were selected in our two previous studies [23,24]. The features of selected markers in higher genetic diversity values such as polymorphic information content (PIC), expected and observed heterozygous, genotypes and allele numbers are presented in Table 1. We conducted PCR analysis using a total reaction
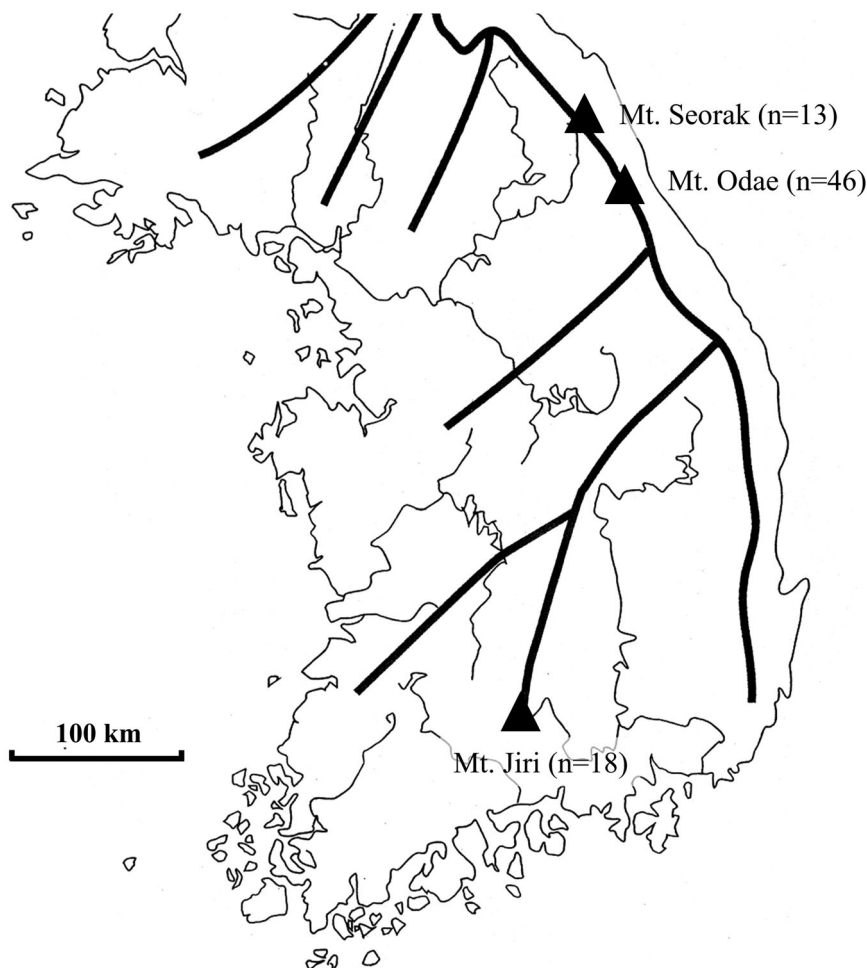

Figure 1. Distribution of 77 wild strains of *Lentinula edodes* in Korea (thin line, river; thick line, mountains).

Table 1. Information of SSR markers and diversity statistics for 100 *Lentinula edodes* strains.

Marker	Forward primer	Reverse primer	Motifs	N_A	H_E	H_O	PIC	HWE χ^2	HWE p-value
RL-LE-001[a]	GTGTGACAGATTACACGGGTC	ACTCAAGTAGGTCCAAGTCGC	(TA)n	25	0.84	0.40	0.82	489.05	0.000***
RL-LE-002[a]	GTAGAGGGATACGCGAAGTAAG	ACAATGGTCGTTCACAGTCG	(AT)n	36	0.88	0.44	0.87	725.68	0.000***
RL-LE-003[a]	CGTTTGCTTGCCCTTTTC	GGAGATACCAACACCACTACTAC	(AC)n	11	0.81	0.02	0.79	477.07	0.000***
RL-LE-004[a]	TGAGCTATGGTGCTACTCCTTC	GCGCCTATATCCGATGGT	(CCA)n	27	0.87	0.21	0.85	697.28	0.000***
RL-LE-006[a]	CTCTGGATAAGAACGCAAGC	CAGTGTTCTCCAGTCCGAGT	(TC)n	25	0.83	0.58	0.81	572.07	0.000***
RL-LE-010[a]	GTAAGCTCATGGTAACAGTGCC	AACTTGGAAGCCTTCTCCC	(AT)n	26	0.75	0.35	0.72	394.67	0.000***
RL-LE-017[b]	GTGCACTGTGCGATTGTTC	CAGCAAGGATGACTCTTGGA	(CA)n	40	0.94	0.18	0.94	1823.55	0.000***
RL-LE-018[b]	CCCACAGGTTTACAGAGTTCCT	GTGGACATCCACCTTTTGTC	(TA)n	17	0.84	0.20	0.82	400.53	0.000***
RL-LE-021[b]	GCTTGAAGAGCGAGTTTGAG	CAAGACACGCTTCGTAGTCA	(AG)n	19	0.71	0.32	0.66	145.61	0.000***
RL-LE-025[b]	TTGGGAGATGCGAGTAGTTC	ATTCAGTCGCTCAGTAGGAGAC	(AT)n	15	0.70	0.29	0.66	252.98	0.000***
RL-LE-026[b]	GATTTGACGCTCACATCCC	CCCCTAAGTATGAGCTTCCGTA	(AG)n	16	0.74	0.51	0.70	126.33	0.000***
RL-LE-028[b]	GAGACGACACGAGGAATTTG	GTCGTTCTCATTGGAGACTCTG	(CA)n	9	0.63	0.12	0.57	218.63	0.000***
RL-LE-033[b]	GACAGAAGAAGGACTTACCAGC	CCAGAGCCCAAGGATAACTT	(CT)n	21	0.75	0.37	0.71	435.69	0.000***
RL-LE-038[b]	CGTTTGAGTGTCAACGGTCT	CATGTCAGACTAGTCAGGGGTC	(AT)n	15	0.77	0.29	0.73	355.74	0.000***
RL-LE-045[b]	ACATCTGAGAGGTCGTACGCT	GTACCGAAGCGAGCAAGTT	(CA)n	13	0.75	0.44	0.71	210.89	0.000***
RL-LE-046[b]	GCACGCAGTGATGAATAGAGAG	ACACTTACGGATTTGGCAGG	(AG)n	10	0.72	0.06	0.68	452.79	0.000***
RL-LE-048[b]	GTGGTGAAGTTACCGACAGG	AGGTGCCCAACTTCTGGT	(GC)n	11	0.63	0.16	0.59	382.95	0.000***
RL-LE-051[b]	ACTCTGCTGCCACTCTTGAC	GACCGTCTCTAGCTTCTTGATG	(CT)n	13	0.62	0.24	0.57	274.91	0.000***
RL-LE-053[b]	CTCAACGTCTCATTCCCTTC	CTCGAGTTGAGGGTGAGGTTAT	(GTT)n	24	0.80	0.51	0.77	203.92	0.000***
RL-LE-059[b]	CGGAGATGTACCAATTCCTG	GCATTCGCCGTCTATACGAT	(TG)n	16	0.74	0.23	0.70	513.50	0.000***

N_A: number of alleles; H_E: expected heterozygosity; H_O: observed heterozygosity; PIC: polymorphic information content; HWE: Hardy–Weinberg equilibrium, ***$p < 0.001$.
[a]Moon et al. (2017).
[b]Lee et al. (2017).

volume of 20 μL containing 2 μL of template DNA, 1 μL of forward and reverse primer (5 pmol) each, 10 μL 2Xi-Taq Master Mix (Intron biotechnology, Seongnam, Korea) and 6 μL of distilled water. The PCR was performed at 95 °C for 3 min; 35 cycles at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s; and finally, 72 °C for 20 min. The size of the PCR product was confirmed using a size fragment analyzer (Advanced Analytical Technologies, Ankeny, IA).

2.3. Data analysis

The PCR products were analyzed using a size fragment analyzer and confirmed by the PROSize 2.0 software (Advanced Analytical Technologies). Based on scored size of the PCR products, the number of alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), and PIC values were calculated using PowerMarker V3.25 [25]. The genetic distance was calculated using the Nei [26] method and the unweighted pair group method with arithmetic mean (UPGMA) dendrogram was constructed using MEGA 7.

A population structure analysis was performed using the model-based software program STRUCTURE 2.3.4 and a model allowing for admixture [27]. The program set a burn-in of 100,000 and a run length of 200,000, which was replicated thrice. The log probability of the data (LnP(D)) was calculated to confirm the population structure of the strains. Although the number of populations (K) was analyzed from 1 to 10, it was not clear as the calculated K value on LnP (D) gradually increased. Therefore, we analyzed the ΔK, proposed by Evanno et al. [28], to determine the optimum K. The strains

sharing more than 70% of ancestry were assigned into one subpopulation [29].

Analysis molecular of variation analysis (AMOVA) was used to assess the genetic differentiation (F_{ST}), and confirmation of the Hardy–Weinberg equilibrium (HWE) was performed using GenAlEx 6.502 [30].

3. Result

3.1. SSR polymorphism

We initially analyzed the information regarding the SSR markers and the diversity statistics of all 100 *L. edodes* strains. We found that the N_A ranged from 9 (RL-LE-028) to 40 (RL-LE-017) with an average of 19. The H_E ranged from 0.62 (RL-LE-051) to 0.94 (RL-LE-017) with an average of 0.76, the H_O ranged from 0.02 (RL-LE-003) to 0.58 (RL-LE-006) with an average of 0.3. The PIC value ranged from 0.57 (RL-LE-028, RL-LE-051) to 0.94 (RL-LE-017) with an average of 0.73. All the markers significantly deviated from the HWE (Table 1).

3.2. Genetic distance and population structure

The 100 *L. edodes* strains were then divided into two groups with two subgroups using the UPGMA dendrogram based on genetic distance through Nei's method [26]. The two groups were Group 1 consisting of only 9 wild strains and Group 2, that was divided into subgroup Group 2-1 containing 58 wild strains (10 in Mt. Seorak, 32 in Mt. Odae, and 16 in Mt. Jiri) and 2 cultivars (SJ102 and SJ103), and subgroup Group 2-2 containing 21 cultivars and 10 wild strains (2 in Mt. Seorak, 7 in Mt. Odae, and 1 in Mt. Jiri) (Supplementary Table S1, Figure 2(A)).
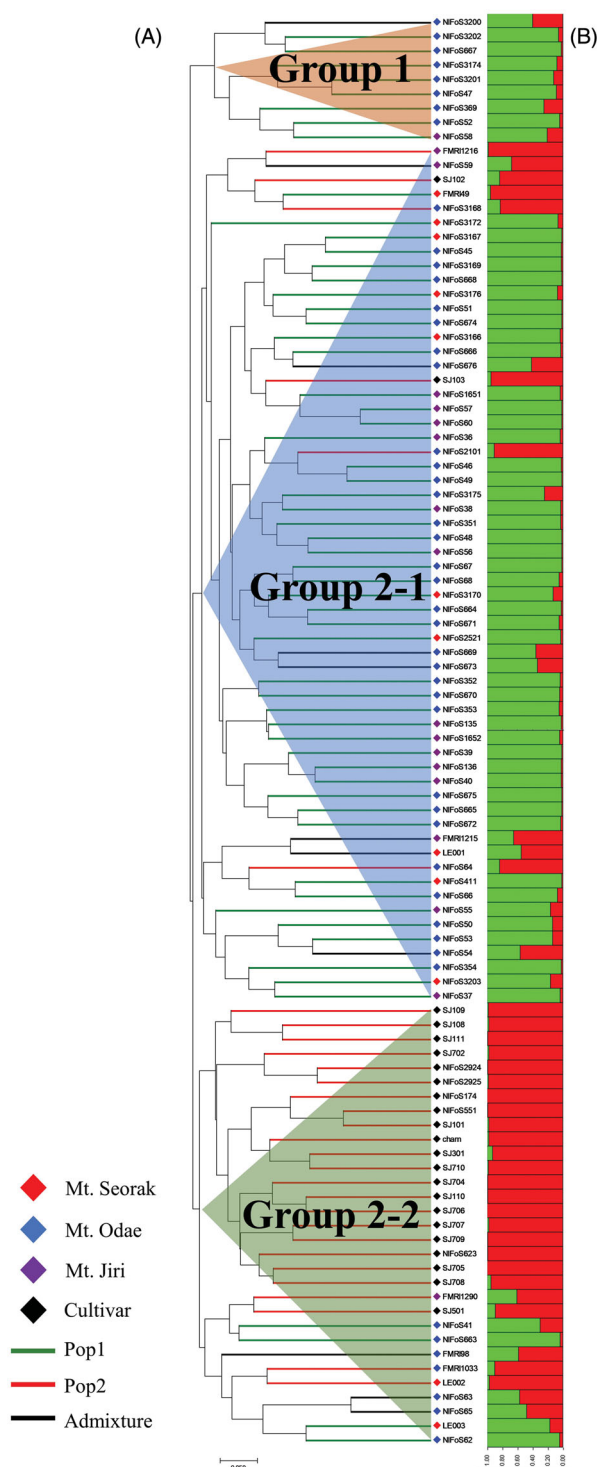
Figure 2. Clustering of distance- and model-based for 100 *Lentinula edodes* strains including 77 wild strains and 23 cultivars using 20 genomic SSR markers. (A) UPGMA dendrogram based on Nei's genetic distance; (B) population structure analysis (green, Pop1; red, Pop2; the strains that did not share more than 70% of ancestry were assigned to admixture).

The log probability of the data (LnP(D)) was calculated to confirm the population structure of the strains. ΔK showed the highest value when K was 2 (Figure 3). Further, 89 strains were assigned into two subpopulations. The subpopulations were divided into Pop1 comprising 59 wild strains, and Pop2 comprising 23 cultivars and 7 wild strains (1

in Mt. Seorak, 4 in Mt. Odae, and 2 in Mt. Jiri). The admixture contained 11 wild strains (1 in Mt. Seorak, 8 in Mt. Odae, and 2 in Mt. Jiri) (Supplementary Table S1, Figure 2(A,B)).

3.3. Analysis of genetic diversity based on the collection regions and types

Among the collection regions, wild strains and cultivars, distance- and model-based analysis was done to compare the genetic diversity. The N_A was the highest for 12 in Mt. Odae, 7 in Mt. Seorak, and 6 in Mt. Jiri, the H_E was the lowest for 0.66 in Mt. Jiri, 0.71 in Mt. Seorak, and 0.72 in Mt. Odae, the H_O was the highest 0.27 in Mt. Odae than 0.23 in Mt. Seorak and 0.22 in Mt. Jiri, and the PIC value was 0.62 in Mt. Jiri, 0.67 in Mt. Seorak and 0.68 in Mt. Odae.

Among the wild strains and cultivars, N_A of the wild strains was 15 and was higher than that of the cultivars (9), and the H_E, H_O, and PIC values of the cultivars (0.76, 0.44, and 0.73) were higher than that of the wild strains (0.73, 0.25, and 0.69), respectively (Table 2).

3.4. Analysis of molecular variation (AMOVA) and Hardy–Weinberg equilibrium based on collection regions and types

The variation was 1% among collection regions, 66% among strains, and 33% within strains. In addition, F_{ST} was 0.01 and gene flow (Nm) was 22.48. Among the wild strains and the cultivars, the variation was 8% between the types, 57% among strains, and 35% within strains, with an F_{ST} value of 0.08, and Nm of 2.98 (Table 3).

All the markers of the wild strains and the cultivars deviated significantly from the HWE. In the collection regions, three markers (RL-LE-004, RL-LE-033, and RL-LE-053) corresponding to samples collected from Mt. Seorak and one marker (RL-LE-004) from the sample from Mt. Jiri were confirmed using HWE, and all markers from the samples from Mt. Odae deviated significantly from the HWE (Supplementary Table S2).

4. Discussion

The SSR markers in this study were analyzed to reveal N_A of 19, H_E of 0.76, H_O of 0.3, and PIC value of 0.73 for 100 *L. edodes* strains including wild strains and cultivars (Table 1). The N_A, H_E, H_O, and PIC values obtained from the study by Lee et al. [23] were 4.9, 0.55, 0.31, and 0.51, respectively. Moon et al. [24] reported the values of 6.8, 0.64, 0.32, and 0.61 for N_A, H_E, H_O, and PIC,
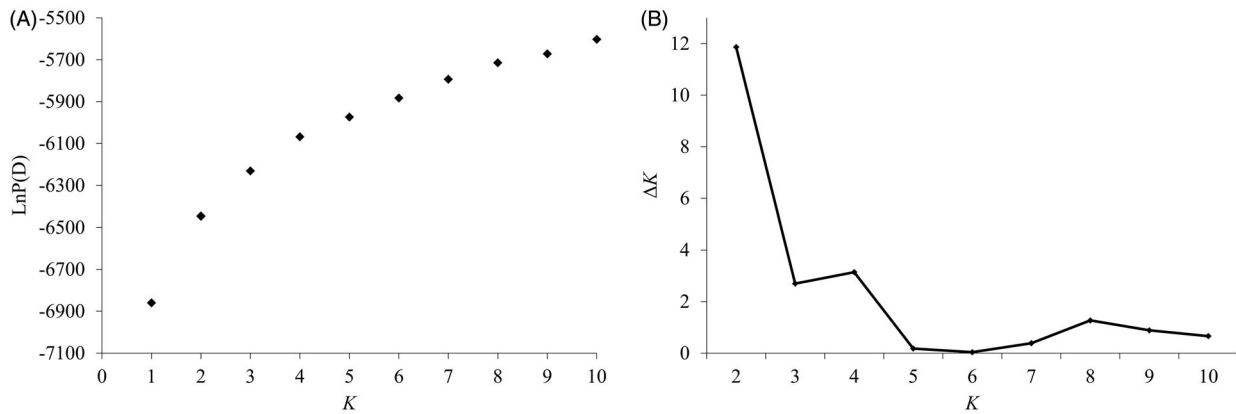
Figure 3. (A) Log probability of the data (LnP(D)) for each K range from 1 to 10; (B) ΔK values calculated as method by Evanno et al. [28].

Table 2. Diversity statistics as determined by collection regions and types.

	N_A	H_E	H_O	PIC
Collection regions				
Seorak	7	0.71	0.23	0.67
Odae	12	0.72	0.27	0.68
Jiri	6	0.66	0.22	0.62
Type				
Wild strains	15	0.73	0.25	0.69
Cultivars	9	0.76	0.44	0.73

N_A: number of alleles; H_E: expected heterozygosity; H_O: observed heterozygosity; PIC: polymorphic information content.

Table 3. Analysis of molecular variance (AMOVA) by collection regions and types.

	df	SS	MS	Est. Var.	%
Collection regions					
Among regions	2	31.30	15.65	0.08	1
Among strains	74	899.29	12.15	4.86	66
Within strains	77	187.50	2.44	2.44	33
Total	153	1118.08		7.38	100
F_{ST}	0.01				
Nm	22.48				
Types					
Among types	1	56.4	56.43	0.63	8
Among strains	98	1183.72	12.08	4.61	57
Within strains	100	286.00	2.86	2.86	35
Total	199	1526.14		8.10	100
F_{ST}	0.08				
Nm	2.98				

df: degrees of freedom; SS: sum of squares; MS: mean of squares; Est. Var.: estimate of variance; %: percentage of total variation; F_{ST}: genetic differentiation; Nm: gene flow; among regions: variation between collection regions; among strains: variation between strains; within strains: variation by difference of molecular markers such as alleles in individual strain.

respectively. Our results showed higher values for H_E and PIC while the H_O value was slightly lower compared to the results of the previous studies [23,24]. The five SSR markers developed by Kim et al. [31] were reported to have H_E of 0.58, H_O of 0.38, and PIC value of 0.53 using cultivars from Korea, China, and Japan, as well as wild strains from Korea. Analysis of these markers showed H_E of 0.53, H_O of 0.38, and PIC value of 0.5 using 10 wild strains collected from Mt. Gariwang and Mt. Jungwang in Korea [32]. The SSR markers used in this study showed higher N_A, H_E, and PIC values

while the H_O value was lower than in that found in the other SSR markers from Korea.

Lentinula edodes in Korea was divided into Group 1 consisting of only 9 wild strains. Group 2-1 contained mainly wild strains along with several cultivars, and Group 2-2 consisted of mainly cultivars with several wild strains by distance-based analysis (Supplementary Table S1, Figure 2(A)). This result was similar to that observed in a previous study by Xiao et al. [33]. The population structure based on the structure model was divided into subpopulation Pop1, which consisted of 59 wild strains, and subpopulation Pop2, which had 7 wild strains and 23 cultivars. The admixture consisted of 11 wild strains (Supplementary Table S1, Figure 2(A,B)). The result of study from Xiao et al. [33] suggested that the spores or the mycelia of the cultivars in the farm might be dispersed into the wild by natural and human activities or maybe the wild strains were used for the development of cultivars in *L. edodes*. The Korean *L. edodes* are also assumed to have influenced each other between cultivars and wild strains. In this study, we showed that the cultivars of Group 2-1 are probably belonged to the cultivars developed using wild strains. However, the wild strains of Groups 2-2 are likely distributed in nature under the influence of the cultivars. The model-based structural analysis of this study, the analyzed strains sharing more than 70% of ancestry were assigned to one subpopulation. Thus, 7 wild strains in Pop2 shared more than 70% of ancestry with cultivars. These results suppose that the wild strains distributed in nature such as distance-based wild strains in Group 2-2 were affected by cultivars.

In this study, H_E and H_O, which are very sensitive to frequency of allele [34] and the PIC value, which is determined by frequency of allele [35] were 0.76, 0.44, and 0.73 in the cultivars and 0.73, 0.25, and 0.69 in wild strains, respectively (Table 2). Therefore, H_E, H_O, and PIC values of the cultivars were higher than those of the wild strains. In several

recent studies, the analysis of 88 wild strains showed H_E of 0.44, H_O of 0.29, and PIC of 0.31 using two SSRs and 68 InDels [13], and the analysis of 89 cultivars revealed H_E of 0.46, H_O of 0.55, and PIC value of 0.38 using 48 SSRs and 249 InDels [36]. Studies conducted in Korea and China have shown that the genetic diversity of *L. edodes* was also higher in the cultivars than in the wild strains. However, Ha et al. [37] reported that the most frequent alleles of A mating type alleles of the *L. edodes* cultivars developed in Korea, China, and Japan were allele A1 and A5. However, these alleles were not investigated in the wild strains collected from Korea. Therefore, stable cultivars previously developed in other countries are expected to be used for the development of *L. edodes* cultivars in Korea. Analysis of AMOVA showed that the variations due to among strains in the collection regions and types were 66% and 57%, respectively, which was the highest attributable to variation in the population. However, the variation of among regions and types was the lowest to 1% and 8%, respectively (Table 3). These results suggest that the *L. edodes* in Korea has a very low genetic variation among the collection sites, cultivars and wild strains. Based on the results of the AMOVA analysis, F_{ST} and Nm values of the collection regions and types were compared. F_{ST} and Nm values between wild strains and cultivars were 0.08 and 2.98, respectively, and the F_{ST} and Nm values among collection regions were analyzed to 0.01 and 22.48, respectively. Therefore, the genetic variation between wild strains and cultivars was higher than that among collection regions in wild strains. In the HWE analysis, *L. edodes* cultivars of Korea significantly deviated from the HWE. Among the collection regions for wild strains, a few markers from the samples from Mt. Seorak and Mt. Jiri were confirmed as HWE (Supplementary Table S2). Non-random mating, migration, mutation, natural selection, and mixture of subpopulation are factors that might lead to deviation from HWE [38]. Therefore, genetic diversity of cultivars was higher than wild strains as it is assumed that the wild strains undergo genetic exchange with other strains less actively than cultivars.

This study revealed the genetic diversity and population structure of wild strains and cultivars of *L. edodes* collected in South Korea. The analysis of genetic diversity and population structure is able to provide important information about the origin and evolutionary features of species. Moreover, it can be valuable in the selection of potential genetic resources for future use [13]. Securing the genetic potential of wild genetic resources with these powerful analyses has been applied as a major tool for improvement of diverse crop traits [12]. The introduction of wild genetic resources is considered as a method for the development of new cultivars with advanced adaptability to the numerical changes by biotic and abiotic stresses [10,11]. In conclusion, the genetic diversity of *L. edodes* cultivars from Korea was higher than that of the wild strains as the mixture of diverse cultivars developed presumably in other countries. However, the use of *L. edodes* wild strains in Korea, which have been never used in the breeding program, will enable the development of novel mushroom cultivars with improved adaptability to biotic and abiotic stress.

Disclosure statement

The authors report no conflicts of interest.

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ORCID

Hyeon-Su Ro 🄳 http://orcid.org/0000-0003-1128-8401

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