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RESEARCH ARTICLE

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Development of Genomic Simple Sequence Repeat Markers for Evaluating Resources of *Armillaira ostoyae* and Their Transferability to *Armillaira gallica*

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ABSTRACT

In this study, we aimed to develop simple sequence repeat (SSR) markers for evaluating resources in *Armillaria ostoyae* and examine their transferability to *Armillaria gallica,* related species. SSR markers were developed using the released *A. ostoyae* whole-genome sequence (GenBank assembly accession: GCA_900157425.1). The SSR regions were analyzed using the MISA (MIcroSAtellite identification tool) program. A total of 2319 SSR loci consisting of 922 (39.76%) mononucleotide, 763 (32.90%) trinucleotide, and 517 (22.29%) dinucleotide motifs were identified. Marker design involved an arbitrary choice of 150 SSR loci, considering motif abundance. A total of 22 strains of *A. ostoyae* were analyzed using the developed markers, and 105 markers were successfully amplified. The mean values of major allele frequency, number of alleles, expected heterozygosity, observed heterozygosity, and polymorphism information content (PIC) values were approximately 5.89, 5.4, 0.541, 0.255, and 0.504, respectively. *A. gallica* was analyzed, and 52 markers (49.5%) were successfully amplified to evaluate the transferability of the developed SSR markers. When these markers were used, the mean values of major allele frequency, number of alleles, expected heterozygosity, observed heterozygosity, and PIC were calculated to be approximately 0.615, 4.3, 0.517, 0.133, and 0.502, respectively. In conclusion, SSR markers were developed using the genome of *A. ostoyae*, and some of these markers exhibited transferability to *A. gallica*. These results can be used for resource evaluation of *A. ostoyae* and *A. gallica*.

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Armillaria ostoyae; SSR marker; *Armillaria gallica*; transferability

1. Introduction

Armillaria, taxonomically belonging to the family Physalacriaceae within the phylum Basidiomycota, encompasses approximately 40 species worldwide [1]. They are white-rot fungi capable of decomposing lignin and parasitizing various coniferous and deciduous trees, woody vines, and even stumps [2].

These fungal species are pathogens of various plants, and their pathogenicity varies depending on the species [3]. Rhizomorphs are subterranean, cord-like structures measuring 1–5 mm in diameter, composed of tightly packed hyphae surrounded by a melanized outer layer that provides protection against environmental stress and facilitates extension into the surrounding soil. This form of vegetative propagation enables *Armillaria* species to establish large genets that can persist in forest ecosystems for centuries or even millennia. Functionally, rhizomorphs facilitate efficient nutrient and water transport over long distances and play a critical role in host root colonization and infection [4–6].

Genotypes derived from vegetative propagation maintain stable habitats and exhibit high longevity, influencing multiple generations of host trees and potentially affecting forest structure and dynamics [7,8]. Additionally, *Armillaria* species can spread over long distances by wind through basidiospores [7]. These two dispersal strategies may affect population genetic structure; therefore, genetic markers are needed to reveal them at different spatial scales [7]. Assessment of the extent to which sexual and asexual reproduction influence population structure and disease transmission can help develop novel pathogen management strategies [9].

Armillaria species are also used in functional foods. Also known as honey mushroom, it has antioxidant [10], antibacterial [11], and anticancer [12] properties, and experiments using mice have revealed its potential to alleviate insomnia [6,13]. Molecular markers are widely used for the management of genetic resources. For example, they are essential tools for identifying varieties [14,15]. Additionally, molecular markers aid in

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the early selection and analysis of population structure [16]. Molecular markers are effective tools for evaluating genetic diversity because they are based on the genotypes of genetic resources and independent of environmental variations. Therefore, resource evaluation using molecular markers is necessary for the efficient utilization of *Armillaria* [17].

With the increasing importance of genetic information and advancements in molecular biology, there is a growing demand for genome analysis and genetic diversity assessments [18]. The main markers used for genetic diversity analysis include single nucleotide polymorphism (SNP), and simple sequence repeats (SSR). SSR consist of 1–6 bp repeat sequences within the genome [19]. While incurring high initial development costs such as sequencing and primer design, SSR loci may possess high polymorphism information content (PIC) and harbor multiple alleles at each locus. Consequently, they are widely used for genetic diversity analysis, pedigree analysis, and population structure studies [20]. Comparative genetics has shown that SSR loci are highly conserved among closely related species, and the collinearity of common markers in comparative maps suggests that markers from one genus or species are present in other related genera/species [21,22]. Therefore, the application of SSR markers developed from one species to another by exploiting transferability, which allows the detection of marker sequences of related species using primer pairs designed based on sequences obtained from one species, has been successfully demonstrated in many species [23]. This approach eliminates the need to develop new markers for each species, making it economically efficient. Among the *Armillaria*, SSR markers were developed using several sequences of *Armillaria mellea*, *Armillaria gallica*, and *Armillaria ostoyae* [24], eight EST-based SSR markers for *Armillaria luteo-virens* were developed through 454 pyrosequencing [25], and 17 SSR markers were developed using 32 single-copy protein-coding genes of 12 *Armillaria cepistipes* samples [7]. In addition, Prospero and coauthors [7] isolated eight polymorphic SSR markers for *A. cepistipes* and confirmed that six markers were polymorphic in *A. gallica*, four in *A. ostoyae*, two in *A. mellea*, and one in *Armillaria borealis*. According to comparative genetics, *A. gallica* and *A. cepistipes* are closely related, with *A. ostoyae* being the next closest relative [26–28]. *A. gallica* is widely distributed in Korea and plays a significant role in forest ecosystems as a decomposer and a pathogen. However, the distribution of *A. cepistipes* is limited in Korea [29]. Therefore, this study focused on *A. galliga* [29]. We developed polymorphic SSR markers for *A. ostoyae* based on whole-genome sequences and tested their transferability to *A. gallica*.

2. Materials and methods

2.1. Armillaria strains

All *Armillaria* strains used in this study were obtained from the National Institute of Forest Science, Korea National Arboretum, and Forest Mushroom Research Institute. The study used 22 of the 40 strains of *A. ostoyae* (Table 1) and 16 strains of *A. gallica* (Table 2) from different locations in Korea. *A. ostoyae* and *A. gallica* were selected based on their *Armillaria* genet [30,31]. Mycelia of these strains were extracted and identified using the ITS1/ITS4 region.

2.2. SSR screen and primer design

We used the genome of *A. ostoyae* registered at NCBI (GenBank assembly accession: GCA_900157425.1), for SSR loci analysis. This Swiss genome was used as a reference for comparative genomic analysis in China [32]; and was utilized for analyzing the viruses in Czech samples [33], and a phylogenetic tree based on the whole genomes of *Armillaria* species showed that it was most closely related to samples from Vermont and Idaho in the United States [34]. We conducted SSR locus exploration using Microsatellite Finder (MISA; https://webblast.ipk-gatersleben.de/misa/), setting the criteria based on SSR motifs as follows: dinucleotide repeats with a minimum of six repetitions, trinucleotide repeats with a minimum of five repetitions, and tetranucleotide repeats with a minimum of five or more repetitions. Considering the ratio of the number of motifs, 150 loci (60 dinucleotide repeats, 80 trinucleotide repeats, and 10 tetranucleotide repeats) were selected using a random number generation program (Random Number Generator: https://www.minzkn.com/random.html) (Table 3). Primers for SSR amplification were designed using Primer 3 PLUS program (https://www.bioinformatics.nl/cgibin/primer3plus/primer3plus.cgi) under the following conditions: primer length of 20 mer, Polymerase Chain Reaction (PCR) product size ranging from 150–200 bp, Melting Temperature (TM) set at 55 °C, and a G/C ratio between 40% and 60%.

2.3. PCR and fragment analysis

Fungal strains were cultured on PDA (Potato Dextrose Agar) medium. Mycelia grown on PDA plates were fully harvested after 2 weeks, rapidly

Table 1. Information on *Armillaria ostoyae* strains used in this study.

No.	Strain name	Collecting location	Best match	Identity (%)
1	Nifos 321[a]	10 Go Nishikagura 1 Sen, Asahikawa, Hokkaido	*A. ostoyae* (KT822292.1)	99.51
2	Nifos 846[a]	Mt. Heibang Goesan-gun, Chungcheongbuk-do	*A. ostoyae* (KT822292.1)	99.75
3	Nifos 848[a]	Mt. Odaesan, Jinbu-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (KT822292.1)	99.4
4	Nifos 1230[a]	Yeonggwimi-myeon, Hongcheon-gun, Gangwon-do	*A. ostoyae* (MG931931.1)	99.47
5	Nifos 1954[a]	Yeonggwimi-myeon, Hongcheon-gun, Gangwon-do	*A. ostoyae* (KT822311.1)	96.26
6	Nifos 1957[a]	Yeonggwimi-myeon, Hongcheon-gun, Gangwon-do	*A. ostoyae* (OK324328.1)	99.75
7	Nifos 2299[a]	770 m, 165, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (MG931931.1)	99.83
8	Nifos 2304[a]	899 m, 164, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (OP787670.1)	99.63
9	Nifos 2305[a]	764 m, 165, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (KT822292.1)	99.64
10	Nifos 2306[a]	126, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (OP787670.1)	99.63
11	Nifos 2307[a]	125, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (KT822292.1)	99.88
12	Nifos 2309[a]	906 m, 164, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (OP688126.1)	100
13	Nifos 2310[a]	1109 m. 125, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (KT822292.1)	99.87
14	Nifos 2311[a]	1061 m, 125, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (OP688126.1)	99.75
15	Nifos 2312[a]	126, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (MG931719.1)	99.58
16	Nifos 2313[a]	124, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (KT822292.1)	99.75
17	Nifos 2318[a]	125, Mt. Jungwhang Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (KT822292.1)	98.02
18	20120925-72[b]	Yongsanbong, Sapyeong 1-gil, Gagok-myeon, Danyang-gun, Chungcheongbuk-do	*A. ostoyae* (OP688126.1)	99.88
19	FMRI 7971[c]	Mt. Balwang, Jinbu-myeon, Pyeongchang-gun, Gangwon-do	*A. ostoyae* (AB510896.1)	99.72
20	FMRI 7687[c]	Yeoju-si, Gyeonggi-do	*A. ostoyae* (MH550355.1)	99.04
21	KA15-0657[b]	Mt. Mahwa, Hongcheon-gun, Gangwon-do	*A. ostoyae* (MG931722.1)	99.71
22	KA17-0855[b]	Mt. Cheongok, Socheon-myeon, Bonghwa-gun, Gyeongsangbuk-do	*A. ostoyae* (MG696210.1)	96.91

The best match is the sequence identified from NCBI.
[a]National Institute of Forest Science.
[b]Korea National Arboretum.
[c]Forest Mushroom Research Institute.

Table 2. Information on *Armillaria gallica* strains used in this study.

No.	Strain name	Collecting location	Best match	Identity (%)
1	Nifos 569[a]	57, Hoegi-ro, Dongdaemun-gu, Seoul	*A. gallica* (MG931780.1)	100
2	Nifos 570[a]	57, Hoegi-ro, Dongdaemun-gu, Seoul	*A. gallica* (KY474051.1)	99.51
3	Nifos 847[a]	Donghae-daero, Sonyang-myeon, Yangyang-gun, Gangwon-do	*A. gallica* (KY474051.1)	99.49
4	Nifos 997[a]	Mt. Joryeong, Yeonpung-myeon, Goesan-gun, Chungcheongbuk-do	*A. gallica* (KY389173.1)	99.75
5	Nifos 1572[a]	163 Mt. Gariwang, Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. gallica* (KY474051.1)	99.39
6	Nifos 2010[a]	Mt. Maebong, Daehwa-myeon, Pyeongchang-gun, Gangwon-do	*A. gallica* (KP162327.1)	99.62
7	Nifos 5063[a]	Mt. Bukhan 375, Daeseomun-gil, Deogyang-gu, Goyang-si, Gyeonggi-do	*A. gallica* (KY474051.1)	99.51
8	FMRI-7088c	N. A	*A. gallica* (KY474051.1)	99.51
9	FMRI-7091c	N. A	*A. gallica* (KY474051.1)	99.75
10	FMRI-7930c	N. A	*A. gallica* (AY213570.1)	99.75
11	FMRI-7937c	Tapdong-gil, Jinbu-myeon, Pyeongchang-gun, Gangwon-do	*A. gallica* (AB716750.1)	99.51
12	FMRI-7135c	N. A	*A. gallica* (AY213570.1)	99.51
13	KA12-1405b	Nari Basin, Ulleung-gun, Gyeongsangbuk-do	*A. gallica* (MW947449.1)	99.02
14	KA13-1053b	Mt. Hwangmae Hapcheon-gun, Gyeongsangnam-do	*A. gallica* (KY474051.1)	99.88
15	KA13-1130b	Gwangneung Forest, Soheul-eup, Pocheon-si, Gyeonggi-do	*A. gallica* (AB510881.1)	99.63
16	KA17-0949b	Baeknokdam, Odeung-dong, Jeju-s	*A. gallica* (MW418538.1)	99.4

N. A: Not Available; the best match is the sequence identified from NCBI.
[a]National Institute of Forest Science.
[b]Korea National Arboretum.
[c]Forest Mushroom Research Institute.

frozen in liquid nitrogen, and ground into powder using a mortar and pestle. The crushed samples were subjected to DNA extraction using the GenEx™ Plant kit (GeneAll, Seoul, Republic of Korea) The extracted DNA was then adjusted to a concentration of 20 ng/μL using distilled water and used as

Table 3. SSR loci selected from the whole genome of *Armillaria ostoyae* (GenBank assembly accession: GCA_900157425.1).

Marker name	SSR motif		SSR primer set (5′→3′)	Product size (bp)
FEL-AO-001	(AC)8	F:	GTAATGGGCACTCGTGAAAC	151
		R:	TGAGATACTGTCAGGGCACA	
FEL-AO-002	(TC)6	F:	CTGCTCGGAAATGATTTTGT	163
		R:	AGCAGCTTGGGGAAGATTAT	
FEL-AO-003	(AT)7	F:	TCAAGGGAGCTATTCGACTG	191
		R:	GCAACATGGGAATTTTTCTG	
FEL-AO-004	(TG)6	F:	CGAGGAGATAGCGAAATTGA	195
		R:	AGCATGAACACCACCCTAAA	
FEL-AO-005	(GT)7	F:	TAAATTCCTTGCTCCCTTCC	163
		R:	GTGAGCCAGAAAAATGTGCT	
FEL-AO-006	(CA)6	F:	TCCATCCATCACCCAATATC	151
		R:	GAGGGAGTATGGATGTCACG	
FEL-AO-007	(AG)7	F:	GATGAGATACGGGAGCAATG	155
		R:	TTACAACCAAGGGACGAGAG	
FEL-AO-008	(AT)8	F:	GCGTCGGTTTGTGTATGATT	183
		R:	TCTAGCCCGACATGTTCAGT	
FEL-AO-009	(TC)6	F:	TCTGATCCTCGGTCCATATC	156
		R:	AGCAGCTGTTTGAGAGCAAT	
FEL-AO-010	(GA)7	F:	GAAGCTCCATCAGCACAGTT	196
		R:	CTTCCTTCAACTTGCATCGT	
FEL-AO-011	(AC)8	F:	CCAGATGCAACCAGAGAACT	176
		R:	TAAATTCCTTGCTCCCTTCC	
FEL-AO-012	(AT)6	F:	CTGTTAGCGTCAAAACGATG	180
		R:	ATGCTATCACCGTGTCCAAT	
FEL-AO-013	(AT)6	F:	AATCTGGGGTACATGAGCAA	165
		R:	ACTCCCGTTTCCTGTCTTTT	
FEL-AO-014	(TG)6	F:	GAGGGAGTATGGATGTCACG	151
		R:	TCCATCCATCACCCAATATC	
FEL-AO-015	(AC)8	F:	GGCACTCGTGGAACTAAGTG	199
		R:	TTGACAATTGTACGCAGTCG	
FEL-AO-016	(AC)6	F:	ATGGGATCAGCCTGAGGTAT	198
		R:	TGGACGGACTTTCTGATGAT	
FEL-AO-017	(CG)6	F:	CGCTTTCCCTTTTTCTTTCT	165
		R:	GTCCAAACAAAAGCAGCAGT	
FEL-AO-018	(CT)6	F:	GTTGCTTGCGGTCAATATCT	166
		R:	GTCGAGAGACGAGCAAACAT	
FEL-AO-019	(TG)10	F:	CTTGCTCCCTTCTGCACTTA	154
		R:	TCATGCTTGAGTTGCCTACA	
FEL-AO-020	(AG)8	F:	GGATATGAATGCGGGATAAG	163
		R:	CCACCATCAGCTCCTTTTTA	
FEL-AO-021	(GT)6	F:	CTTGCTCCCTTCTGCACTTA	188
		R:	CCTGAGGAGAAATGTCATGG	
FEL-AO-022	(GT)8	F:	ATCGCGTTGCATTACTTAGC	157
		R:	GCATGAACAACACACCAAAA	
FEL-AO-023	(AC)7	F:	CTATCACTGGATGGCCTCTG	188
		R:	CAGCTGATACTTGGCACTGA	
FEL-AO-024	(GT)8	F:	ATCGCGTTGCATTACTTAGC	161
		R:	GCCTAGCGTGAACAACACTC	
FEL-AO-025	(GT)8	F:	ATCGCGTTGCATTACTTAGC	168
		R:	AAGCATGGCCTAGTGTGAAC	
FEL-AO-026	(AG)7	F:	CGGGAAAACAAACAAAACAG	175
		R:	CTTGTTTCCAAGGGCAGATA	
FEL-AO-027	(AC)6	F:	GCGTGAACAACACCCTAATC	153
		R:	ATCGCGTTGCATTACTTAGC	
FEL-AO-028	(GT)10	F:	ATCGCGTTGCATTACTTAGC	198
		R:	ATTACCGAGCACATCATCGT	
FEL-AO-029	(AC)8	F:	ACTAAGCGCGACCTAGTGTG	171
		R:	ATCGCGTTGCATTACTTAGC	
FEL-AO-030	(AC)8	F:	GTAACGGGCACTCATGAAAC	189
		R:	ATCGCGTTGCATTACTTAGC	
FEL-AO-031	(AT)6	F:	ATGGCGAGGTAGGTTTTTCT	152
		R:	ACAAAGACCCCTCCATTCTC	
FEL-AO-032	(GT)7	F:	TGCCGCATTGCATTATTTAG	187
		R:	TTATGGGCACTTGTGAAACA	
FEL-AO-033	(CT)6	F:	ACTGAGTTTGCTGGTTGAGC	170
		R:	GGGAGAATGTTCAGCAGATG	
FEL-AO-034	(GT)8	F:	TTCCTTGCTCTCTTCCACAC	198
		R:	TAGAAATGGATACCGGCAGA	
FEL-AO-035	(AT)6	F:	TTCAAGTGAGCGCTATGTCA	192
		R:	CGATGGATCAACCCAGTAAG	

(*Continued*)

Table 3. Continued.

Marker name	SSR motif		SSR primer set (5′ → 3′)	Product size (bp)
FEL-AO-036	(AC)6	F:	GTAACGGGCACTCATGAAAC	185
		R:	ATCGCGTTGCATTACTTAGC	
FEL-AO-037	(TG)12	F:	TGCAGTCACGTTGCATTATT	175
		R:	GTGGCCTAGTGTGAACAACA	
FEL-AO-038	(TG)8	F:	ATCGCGTTGCATTACTTAGC	154
		R:	CGTGAACAACACCCTAAACC	
FEL-AO-039	(GT)6	F:	GCCACATTTGTAATCCATCC	179
		R:	ATGATCAATGGGTCACTGCT	
FEL-AO-040	(AG)7	F:	ACGCGATTAAGTAGCAGTGG	182
		R:	TTGAATGCTCAGAGGGACAT	
FEL-AO-041	(AC)7	F:	GCCATTGACTGCTCTCTGAT	162
		R:	TAAACTCCTTGCTCCCTTCC	
FEL-AO-042	(GA)6	F:	ATAGTTGTCGACCTCCGTGA	193
		R:	TGCTACATGTACGGCACACT	
FEL-AO-043	(CG)7	F:	GGCGTCAGTATCTGGGTATG	170
		R:	ACACCAAATTGCAAGAAAGC	
FEL-AO-044	(GA)6	F:	CCCGTGAATATGACGTAACC	151
		R:	TCCGCATTTTCTAAGGGACT	
FEL-AO-045	(AC)9	F:	CAAGGCTGGTAATGAGCACT	158
		R:	GATACTGTCAGGGTGCAAGC	
FEL-AO-046	(AT)6	F:	GCATGTAGGTTCTGGATTGG	188
		R:	TCTCGCGATATCTCCAACAT	
FEL-AO-047	(AG)7	F:	GAGGGTATCCACCGAAAAAT	154
		R:	CTCGCATTTGAAGCTCTGAT	
FEL-AO-048	(GT)8	F:	GATCGCGTTGCATAACTTAG	163
		R:	GGCCTAGCATGAACAACACT	
FEL-AO-049	(CG)6	F:	CACCACTTGTGACTCACGTC	177
		R:	GAATCTCCCATGACGAACAT	
FEL-AO-050	(TC)6	F:	AGCGAGATCCATCACAGAAC	176
		R:	ATTTGGGCAAATCTACACGA	
FEL-AO-051	(AC)7	F:	AGCAAACAAGACCTCCATTG	171
		R:	TTCCTTGCTCTCTTCCACAC	
FEL-AO-052	(AG)6	F:	CGGCAAAAGATATTGGGTAA	150
		R:	GTCGCTCAGGCAAGATACTG	
FEL-AO-053	(TC)9	F:	CCACTAGGTGCTGAAGGGTA	175
		R:	GCGATACGGTGATAGGTCAG	
FEL-AO-054	(CA)8	F:	CTGACACTTTCCACGAGCTT	153
		R:	CTGTGGAGCTCTTTCATGCT	
FEL-AO-055	(AG)6	F:	CTGATTGATTGGACGGACTC	200
		R:	TTGTCTTCCTTTTGGCATGT	
FEL-AO-056	(TC)7	F:	AACAATGTAGAGGCGGTTTG	170
		R:	ATGCCATCTATCAAGGTTGG	
FEL-AO-057	(AC)6	F:	GTCCCATGTCTCTTTCGATG	174
		R:	CATAAGAAGTGGTCGTGCAA	
FEL-AO-058	(AT)6	F:	TTCCATGACACAAGCACATC	181
		R:	TCCAAAATTCGGACCTTACA	
FEL-AO-059	(TC)8	F:	AGCGTCTCTCCTTGTCTTCA	173
		R:	TGTTTCGTATAGGGGTCGAG	
FEL-AO-060	(AG)6	F:	GAAGGGAATCATGACGAGTG	163
		R:	TTCCCTTCTGTGCTATTCCA	
FEL-AO-061	(GAC)5	F:	TCTATCACCGGCTTCTTGTC	191
		R:	CCGCGATACATCATTGAGTT	
FEL-AO-062	(TGG)6	F:	ATGGATAGCAGGTCGATGAG	197
		R:	AGATGCTGGTAGGCACAAAA	
FEL-AO-063	(CTA)5	F:	TCATCGGAGCGTAAGTCTCT	199
		R:	ACATTCACATTCGGATCCAT	
FEL-AO-064	(CGT)5	F:	TCCAATATGTCCAGCTCCAT	161
		R:	CAGCGTACCGTCTCTTTCAT	
FEL-AO-065	(CAT)5	F:	AATAGGGTTGCCTAGGGTTG	181
		R:	GTTTTGGTGTTGGTGTTGGT	
FEL-AO-066	(GAC)5	F:	AGGCACTCAGAACGTCGTAG	174
		R:	GACGGGCATTAGAGTTTCAA	
FEL-AO-067	(AGA)5	F:	ATTAGCGACAGGGAGGAGAT	170
		R:	CGAGCAAGCTCTGTATCCAT	
FEL-AO-068	(TGG)6	F:	CGTTAGGATCATCTGGGTCA	196
		R:	GATCTCACCGAATCTGGATG	
FEL-AO-069	(CAA)5	F:	AGAATTGCAAAACCGATACG	187
		R:	ATGATTAGCGTCGGTCTGTC	
FEL-AO-070	(CAT)5	F:	ATCGACGCATGACTCAAAGT	164
		R:	AAATATAGCGGACCATGTCG	
FEL-AO-071	(TCC)5	F:	GGGTGGAGGTCAAAGTATCC	192
		R:	CTATGACGACTCGCTGGATT	

(*Continued*)

Table 3. Continued.

Marker name	SSR motif		SSR primer set (5′→3′)	Product size (bp)
FEL-AO-072	(CTC)6	F:	ATTCCGTTTCTCCTTGCTCT	191
		R:	GTATTTGCGTGGGTACCTGT	
FEL-AO-073	(ACG)5	F:	ATGGTATTGGGGAGGAAAAG	181
		R:	TGTGTAGAAAATCCGGCAAC	
FEL-AO-074	(CAA)8	F:	GACGATTGTCGGTGTAAAGC	195
		R:	CTGGGATAGGAAAAGGTCGT	
FEL-AO-075	(CTC)5	F:	CAGGTACATTGGGTCGAGAT	193
		R:	TCATTTTGAAACTCCCTGCT	
FEL-AO-076	(GAC)6	F:	TGTCTCCTCCAATGACGATT	158
		R:	ATATCGTTGCGAGAGGTGAT	
FEL-AO-077	(CGT)5	F:	CCCATGGATCATTAATCTGC	185
		R:	TCCGTGCTTACCTTGTCTTC	
FEL-AO-078	(TGG)5	F:	TTAACCATGATGCTCGAAGG	192
		R:	CCCAACTTCTTTCACCCTTT	
FEL-AO-079	(GAC)7	F:	TGAAGAGAAGTCGGGAGTTG	195
		R:	GTATCCAGGGGATTCGAGAG	
FEL-AO-080	(CAG)7	F:	CTCTTTCAACCACCACCATC	182
		R:	CATTTTCCTTCACAGGTTGG	
FEL-AO-081	(CGT)9	F:	ATGGCCGCTGGTAATACATA	157
		R:	TTCATTGAAGAGCTGGTTCC	
FEL-AO-082	(TCC)6	F:	GAGAATGAGCCCACCAATTA	182
		R:	CGGTTTGAGTATGGGAGTGT	
FEL-AO-083	(ATG)6	F:	CAAAAGAGCCAAAGGAGTGA	177
		R:	CCGGACGGAAATCATACATA	
FEL-AO-084	(CGT)6	F:	GAGATCCTTAGACGCGGTTT	165
		R:	ATCGCCTCATCCTCCTCTAT	
FEL-AO-085	(CAC)5	F:	GACGGCTGATAAGACAGTGG	185
		R:	CGGTATGATCTGCTCTCGTT	
FEL-AO-086	(ACT)6	F:	ACTTAATAGGGACCGCTTGC	183
		R:	TGACGCGAATACCCTTACAT	
FEL-AO-087	(GGT)6	F:	AAGGAGCGAGAATGGAACTT	165
		R:	ACCACGCTTCATGAGAAGAA	
FEL-AO-088	(ATC)6	F:	CAATTGCGTGGGATTTCTAT	185
		R:	CAAGGTGGGAAGAGCCTTAT	
FEL-AO-089	(TGG)6	F:	AGGAAAGGCTGATGGGATAC	195
		R:	ATGATGTGAACCGGTTTGAG	
FEL-AO-090	(GAA)5	F:	AAGCATTGGAAGAAGAAGCA	199
		R:	CAGTCTCGGATGACTCTGGT	
FEL-AO-091	(ACG)5	F:	CAGCGATATCAGGGTCAATC	181
		R:	GGCCCTCCATCAGAGTAAAG	
FEL-AO-092	(ACC)5	F:	ATGTGAACGGTCCGATATTC	171
		R:	GGGATTGACGGTGGTAAGTA	
FEL-AO-093	(TCC)5	F:	GCAGCTTGCTTCTTCTTGAG	195
		R:	GCCACACGTTTCCAATATCT	
FEL-AO-094	(GAC)5	F:	CCACGTTCAAAAGTTCGAGT	181
		R:	GAAGCGTGCGGAATAAATAA	
FEL-AO-095	(CCG)5	F:	CGCCATATAGAGCAACCACT	199
		R:	CATTACCCTTCGGCTTCTTT	
FEL-AO-096	(CAC)5	F:	GAAGAGGGTGAGGAGGATGT	162
		R:	ACTGGAGGGGACTGGTAGAG	
FEL-AO-097	(ACG)5	F:	AACGCTGATAGACGCTTGAC	188
		R:	TTCTACCATTGAACCCGGTA	
FEL-AO-098	(CGC)5	F:	ATCTGCAGAGGCTTGTTCAC	196
		R:	TCGTAGTGATTGGTGCACTG	
FEL-AO-099	(CGT)5	F:	AAATCATAGCCGTGATTGGA	150
		R:	ATGACGACGCTATTCTTTGC	
FEL-AO-100	(GGA)5	F:	GACCCTGGATGATTGCATTA	192
		R:	TCGTGGTTAAGGTGCAAGAT	
FEL-AO-101	(TCG)5	F:	AGCGTACCTTTGTCAACGTC	168
		R:	TGGCATGAAAGTCTTCATCA	
FEL-AO-102	(TCG)5	F:	TCGGTGATCCAATCTCTCTC	162
		R:	GGTGGCGAGAAGTAGACGTA	
FEL-AO-103	(AAT)5	F:	GCATGGAGTCTCAGAGGAAG	195
		R:	GCTCGAGTTGAACCTTCTCA	
FEL-AO-104	(TCA)6	F:	TGAATGCCCATCAAGGTACT	170
		R:	ACATCCGTCCATGCAGTAAT	
FEL-AO-105	(AGT)5	F:	TCCTGTATGCGTAAGGGGTA	197
		R:	GCAATACCTTGCTTGTCGAT	
FEL-AO-106	(CAT)5	F:	CCGTTCTTTCATCAATGTCC	190
		R:	CAGTGTGGGAAGTTGGAGTC	
FEL-AO-107	(TCT)9	F:	TGCACTCACACTTGGCATAC	183
		R:	AGATGAAGAGTACGCGATGG	

(*Continued*)

Table 3. Continued.

Marker name	SSR motif		SSR primer set (5′ → 3′)	Product size (bp)
FEL-AO-108	(TTC)7	F:	ACATCCATAGCGTCGTCAGT	183
		R:	CGGAGATCGAGACTTCAAGA	
FEL-AO-109	(CGG)5	F:	GAACGTGGGCTGAGACTCTA	168
		R:	TGACCCAAGCTCAAGGATTA	
FEL-AO-110	(CAT)5	F:	CTGGATTGCCTTATCACCAC	175
		R:	GTGACATGAGGGTGAATTGG	
FEL-AO-111	(TCG)5	F:	GAACCACCATTGGTTACGAG	175
		R:	ATCCATTTGGCATTGAACTG	
FEL-AO-112	(TCA)6	F:	CTCTGCCTGCACTCTTGATT	157
		R:	AGTTGCAGCGTGGAAGTTAC	
FEL-AO-113	(TCC)5	F:	CCATTCTTCGGGAACAACTA	198
		R:	TGGCTATCGCTAATTCGTGT	
FEL-AO-114	(CTA)5	F:	CTCTGCTCCACTTCAGGTGT	197
		R:	CATGGGTTGGAGACCAATAA	
FEL-AO-115	(CTG)5	F:	TCGGATACTCAGGCTCCATA	161
		R:	TCTCTCGGGACAGATGACAC	
FEL-AO-116	(CCG)7	F:	ATACCTGCCCTTCCATTACC	191
		R:	GCGTCTTCTTCTGAGCTGAC	
FEL-AO-117	(CAT)9	F:	CAACGTTCTCAACCGTTTTC	165
		R:	GGCTAGATTTCTACGCCACA	
FEL-AO-118	(CTC)5	F:	GCACTGGACCATGAATTCTC	179
		R:	TGATACAATTGCCGAGGAAC	
FEL-AO-119	(ATC)5	F:	TGGAAAGGCCCCTATCTATC	173
		R:	TGCCTCGGTACCACTATCTC	
FEL-AO-120	(ATG)5	F:	TTGGGTAGCGAAGTATGGAG	168
		R:	TATGTCCTGTTGGAGGATCA	
FEL-AO-121	(GTG)5	F:	TGCACACCAGCTAAGATGAA	153
		R:	CCTGAAACAACCCCAGTATG	
FEL-AO-122	(GAC)6	F:	TGACAAGACGATGGATGATG	188
		R:	CGACAAGGAACGAACAACTT	
FEL-AO-123	(GTG)7	F:	TGCAAGAAAATGTCGATACG	164
		R:	CAAAACGGTATGAGCCTTTG	
FEL-AO-124	(ACC)5	F:	CGTCCAGCCTACTTGAATGT	189
		R:	CAATAATGGGACGAAACGAC	
FEL-AO-125	(TGG)6	F:	CGATGTTCGAATCTCCTGAC	153
		R:	ACGAGAGTTCCCATGTGTGT	
FEL-AO-126	(CAC)7	F:	TCAACGTAGAGAGGGAGCAG	197
		R:	CATCTCGTCGTCGTCTTTCT	
FEL-AO-127	(TGA)7	F:	TGGAGGTCAGGGATGATCTA	159
		R:	GGGATTCTTCTGGCTCCTTA	
FEL-AO-128	(ATG)6	F:	GGGTGTGTGAGTGGTTTAGC	158
		R:	CTGTCGATGACACTGATCCA	
FEL-AO-129	(GTG)6	F:	AGGTGTGGATCGTAGGGAGT	160
		R:	GTTGCCACTGAGAACAAAGG	
FEL-AO-130	(ACA)5	F:	GCTCAAGCGAGAGAGAGAAG	190
		R:	CGTTTGAGGAGGAGAAGGAT	
FEL-AO-131	(GTC)5	F:	GTTTCCCGCACTTCTCTTCT	175
		R:	AACTGCAGAGACACCGAGAC	
FEL-AO-132	(GCA)6	F:	GCATATGGCAAGTGTTCCTT	160
		R:	GTAGTGGTTGGCCATGAGAG	
FEL-AO-133	(GAA)6	F:	CGACGACGAAGATGATACAG	199
		R:	CAGCATCGATTTCCTGTTCT	
FEL-AO-134	(AGG)7	F:	GCATCGGAGAACATCTCATC	164
		R:	GTTGCCGTCTAGATCGATTG	
FEL-AO-135	(TTC)5	F:	AATGCACAAAGCTGGTTCTC	190
		R:	GAAACCGTACCACCAAAGTG	
FEL-AO-136	(TGC)5	F:	CTGGTGGTGTTGGTAGAAGG	174
		R:	CTTCGTTTGGACAGTCAGGT	
FEL-AO-137	(GAT)5	F:	CACGACGAGTCCAGAAAGTT	163
		R:	TGGACTTCCAGAAACGCTAC	
FEL-AO-138	(TTC)6	F:	GTATCTCCGCAGTGAGGAAC	193
		R:	GCAAACACCATCAAACCTCT	
FEL-AO-139	(TCC)7	F:	GTCGCTCCTTCGACTACTCA	166
		R:	GCTTGGCAAAGGTTATTTGA	
FEL-AO-140	(TCA)8	F:	GTGCGTTTCTTCAAGGTCAT	185
		R:	GCGAGAATTCAATGTTCAGG	
FEL-AO-141	(CATT)5	F:	GTCATGATGCCTGCAGTAGA	189
		R:	ACCTTGATTTGCCCAGATTT	
FEL-AO-142	(GGTA)7	F:	AAGGAAGATGTGGTGACAGC	168
		R:	GTTCGTTCGTCAAGAGGAGA	
FEL-AO-143	(TATC)6	F:	TTAATTAGGCGGCACAGAAC	192
		R:	CTGAGGCAAGCATCAAGATT	

(*Continued*)

Table 3. Continued.

Marker name	SSR motif		SSR primer set (5′→3′)	Product size (bp)
FEL-AO-144	(GGTA)5	F:	GGAATTGTCAGGGTGTTGAG	154
		R:	CCACACACTGCCGTGTAATA	
FEL-AO-145	(TTCC)5	F:	GTCGCGGTACAAAGAGAAGA	152
		R:	GTAGCTGGATCCTTGTTGGA	
FEL-AO-146	(ACCT)5	F:	TCATCCATCAGGGTCTGTCT	156
		R:	GCTTCGATCTTGATGAGGAG	
FEL-AO-147	(GGTA)5	F:	TGAAGTTGGACTTTCGCTGT	195
		R:	GCAAGTGATGGTGGTCATGT	
FEL-AO-148	(TAGA)5	F:	ATAAGCATGGGCTGATCAAA	200
		R:	GGGTATTGCTTATTCCACCA	
FEL-AO-149	(CTTC)6	F:	CTTTTAAGGAGCCACAACGA	165
		R:	AAGCCGGCATTTATTACCTC	
FEL-AO-150	(CTTC)5	F:	CGGACGTCGTTATGTTTCTT	172
		R:	CCCACAAAACAGCCAGAATA	

template DNA for PCR reactions. The PCR reaction mixture was prepared by mixing 15 µL of GainBlue™ Hot Start Master Mix, 2× (Gainbio, Daejeon, Republic of Korea), 3 µL of each forward and reverse primer (5 pmol), 10.5 µL of distilled water, and 1.5 µL of DNA, resulting in a total volume of 30 µL. The PCR conditions involved pre-denaturation at 95 °C for 3 min, followed by denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 20 s. This process was repeated for 35 cycles, followed by a final extension at 72 °C for 20 min. The amplified PCR products were electrophoresed on 2% agarose gel to confirm amplification. Subsequently, fragment sizes were analyzed using the PROSize 2.0 software (Advanced Analytical Technologies, Ankeny, IA, USA) on a Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA, USA). Transferability was tested by genotyping 16 *A. gallica* isolates using 150 SSR loci.

2.4. Data analysis

Using the data obtained from the Fragment Analyzer, genetic diversity was estimated based on the following parameters. Major allele frequency (M_{AF}) indicates the relative frequency of alleles, expected heterozygosity (H_E) represents the expected level of heterozygosity calculated under genetic equilibrium conditions, and observed heterozygosity (H_O) reflects the observed level of heterozygosity calculated from the actual populations. PIC is commonly used to measure genetic diversity arising from polymorphisms in the presence of two or more alleles at specific loci in a population [35]. PIC represents the information content of the DNA markers, whereas the number of alleles (NA) reflects the average number of observed alleles per locus [35]. The parameters mentioned above were analyzed using Power Maker V3.25 program. Based on these results, an unweighted pair group method with arithmetic mean (UPGMA) tree was constructed using the shared allele method.

3. Results and discussion

3.1. SSR distribution

Using the *A. ostoyae* genome, 2319 SSR loci were identified. Among these, mononucleotide motifs were the most frequent (39.8% of SSR loci). Among the mononucleotide motifs, T (40.8%), A (37.8%), and C (10.9%) were the most abundant in the same order [36]. This pattern is similar to previous studies on SSR loci in nine fungal species (*Aspergillus nidulans*, *Cryptococcus neoformans*, *Encephalitozoon cuniculi*, *Fusarium graminearum*, *Magnaporthe grisea*, *Neurospora crassa*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe, Ustilago matdis*), where mononucleotide motifs were the most abundant and A/T repeats accounted for >80% of the repeats [36]. Similar findings have been reported in other fungal species, such as *Schizophyllim commune*, *Copricopsis cinerea*, *Laccaria bicolor*, Pleurotus *ostreatus* where the proportion of A/T repeats is overwhelmingly high [37–40]. Following mononucleotide motifs, trinucleotide motifs were the next most abundant, with 763 loci (32.90%). Compared to previous studies, trinucleotide motifs were found to be abundant in *N. crassa* with 4,084 motifs, in *M. grisea* with 1,573 motifs, and in *U. maydis* with 865 motifs, following mononucleotide motifs [41]. Similarly, trinucleotide motifs are prevalent in *S. commune*, *C. cinerea*, *L. bicolor*, and *P. ostreatus* [37–40]. Trinucleotide motifs were the second most abundant, accounting for 33.86% of the total in *Leptosphaeria maculans*, the causal agent of blackleg disease [42], Similarly of *A. bisporus* SSR loci revealed trinucleotide motifs as the most abundant, with 898 loci identified, reflecting a pattern similar to the findings of this study [43]. The third most abundant motif was dinucleotides, with 517 loci

Table 4. Diversity statistics from selected 105 SSR markers used for analyzing 22 *Armillaria ostoyae* strains.

Marker	M_{AF}	N_A	H_E	H_O	PIC
FEL-AO-001	0.500	6	0.685	0.571	0.650
FEL-AO-002	0.300	6	0.751	0.300	0.709
FEL-AO-003	0.286	9	0.800	0.143	0.773
FEL-AO-004	0.368	8	0.796	0.053	0.774
FEL-AO-006	0.818	4	0.318	0.000	0.302
FEL-AO-009	0.523	5	0.632	0.636	0.576
FEL-AO-010	0.528	4	0.616	0.056	0.552
FEL-AO-012	0.571	2	0.490	0.000	0.370
FEL-AO-013	0.667	2	0.444	0.000	0.346
FEL-AO-014	0.795	6	0.358	0.045	0.347
FEL-AO-015	0.545	6	0.617	0.182	0.564
FEL-AO-017	0.500	5	0.667	0.000	0.622
FEL-AO-018	0.432	6	0.669	0.136	0.612
FEL-AO-024	0.455	6	0.704	0.500	0.662
FEL-AO-025	0.595	6	0.571	0.190	0.518
FEL-AO-027	0.405	7	0.748	0.333	0.715
FEL-AO-029	0.444	6	0.690	0.278	0.642
FEL-AO-030	0.262	9	0.799	0.286	0.770
FEL-AO-031	0.700	6	0.485	0.150	0.460
FEL-AO-032	0.342	7	0.756	0.211	0.719
FEL-AO-033	0.471	9	0.720	0.176	0.692
FEL-AO-035	0.571	9	0.639	0.286	0.617
FEL-AO-036	0.500	4	0.546	0.100	0.444
FEL-AO-037	0.286	10	0.819	0.619	0.796
FEL-AO-038	0.795	5	0.347	0.364	0.322
FEL-AO-039	0.341	7	0.788	0.318	0.760
FEL-AO-042	0.667	6	0.523	0.048	0.493
FEL-AO-043	0.762	2	0.363	0.000	0.297
FEL-AO-045	0.273	6	0.778	0.455	0.741
FEL-AO-046	1.000	1	0.000	0.000	0.000
FEL-AO-048	0.909	3	0.169	0.091	0.163
FEL-AO-049	0.250	10	0.850	0.455	0.834
FEL-AO-050	0.625	3	0.531	0.100	0.468
FEL-AO-052	0.571	4	0.598	0.238	0.546
FEL-AO-053	0.705	6	0.485	0.318	0.465
FEL-AO-054	0.523	8	0.683	0.364	0.657
FEL-AO-055	0.679	5	0.508	0.071	0.478
FEL-AO-057	0.357	7	0.732	0.810	0.690
FEL-AO-058	0.381	7	0.730	0.333	0.688
FEL-AO-060	0.682	4	0.479	0.091	0.427
FEL-AO-063	0.886	2	0.201	0.045	0.181
FEL-AO-064	0.750	3	0.394	0.091	0.344
FEL-AO-066	0.600	7	0.613	0.000	0.594
FEL-AO-067	0.619	5	0.576	0.000	0.544
FEL-AO-070	0.295	10	0.822	0.773	0.801
FEL-AO-071	0.295	11	0.790	0.682	0.762
FEL-AO-073	0.568	6	0.632	0.182	0.602
FEL-AO-075	0.619	3	0.526	0.190	0.455
FEL-AO-076	0.310	9	0.814	0.429	0.791
FEL-AO-077	0.607	4	0.556	0.143	0.499
FEL-AO-078	0.533	6	0.647	0.200	0.606
FEL-AO-079	0.477	7	0.714	0.591	0.685
FEL-AO-080	0.310	10	0.819	0.857	0.797
FEL-AO-081	0.275	10	0.826	0.500	0.805
FEL-AO-082	0.636	4	0.524	0.273	0.465
FEL-AO-083	0.614	4	0.556	0.227	0.503
FEL-AO-084	0.295	9	0.823	0.364	0.803
FEL-AO-086	0.905	4	0.178	0.095	0.172
FEL-AO-089	0.786	4	0.366	0.190	0.346
FEL-AO-092	0.786	6	0.373	0.238	0.361
FEL-AO-093	0.682	6	0.502	0.545	0.471
FEL-AO-094	0.976	2	0.046	0.048	0.045
FEL-AO-095	0.750	3	0.394	0.045	0.344
FEL-AO-099	0.955	2	0.087	0.000	0.083
FEL-AO-100	0.475	5	0.635	0.300	0.568
FEL-AO-101	0.909	3	0.168	0.091	0.160
FEL-AO-102	0.667	2	0.444	0.000	0.346
FEL-AO-103	0.625	6	0.570	0.400	0.538
FEL-AO-104	0.524	4	0.591	0.571	0.515
FEL-AO-106	0.909	2	0.165	0.000	0.152
FEL-AO-107	0.690	5	0.491	0.429	0.460
FEL-AO-109	0.452	5	0.646	0.333	0.580
FEL-AO-110	0.864	3	0.244	0.091	0.228
FEL-AO-111	0.659	4	0.510	0.182	0.461
FEL-AO-112	0.614	7	0.590	0.318	0.564
FEL-AO-113	0.682	6	0.496	0.364	0.459
FEL-AO-114	0.600	4	0.545	0.100	0.476
FEL-AO-115	0.810	4	0.331	0.095	0.313
FEL-AO-116	0.568	6	0.631	0.182	0.600

(*Continued*)

Table 4. Continued.

Marker	M_{AF}	N_A	H_E	H_O	PIC
FEL-AO-117	0.452	6	0.713	0.524	0.675
FEL-AO-118	0.773	2	0.351	0.091	0.290
FEL-AO-119	0.619	4	0.505	0.095	0.418
FEL-AO-120	0.738	4	0.426	0.286	0.394
FEL-AO-121	0.500	3	0.522	0.182	0.407
FEL-AO-122	0.386	6	0.707	0.545	0.657
FEL-AO-124	0.225	8	0.826	0.300	0.803
FEL-AO-125	0.433	5	0.709	0.200	0.665
FEL-AO-127	0.857	2	0.245	0.095	0.215
FEL-AO-129	0.452	6	0.693	0.381	0.647
FEL-AO-130	0.591	6	0.582	0.636	0.532
FEL-AO-131	0.909	2	0.165	0.000	0.152
FEL-AO-132	0.909	3	0.169	0.000	0.163
FEL-AO-133	0.432	5	0.635	0.636	0.565
FEL-AO-134	0.714	5	0.467	0.095	0.444
FEL-AO-135	0.818	5	0.319	0.045	0.304
FEL-AO-136	0.886	4	0.210	0.136	0.202
FEL-AO-137	0.786	4	0.366	0.143	0.346
FEL-AO-139	0.295	8	0.822	0.455	0.800
FEL-AO-140	0.477	6	0.682	0.591	0.637
FEL-AO-142	0.952	2	0.091	0.000	0.087
FEL-AO-143	0.325	10	0.808	0.600	0.784
FEL-AO-145	0.500	6	0.670	0.591	0.627
FEL-AO-146	0.658	5	0.533	0.158	0.503
FEL-AO-147	0.238	8	0.841	0.476	0.822
FEL-AO-148	0.977	2	0.044	0.045	0.043
MAX	1.000	11.000	0.850	0.857	0.834
MIN	0.225	1.000	0.000	0.000	0.000
Mean	0.589	5	0.541	0.255	0.504

M_{AF}: major allele frequency; N_A: number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; PIC: polymorphism information content.

(22.29%), followed by tetranucleotides with 83 loci (3.58%) and hexanucleotides with 29 loci (1.25%). Pentanucleotide repeats were the least abundant, with only five loci (0.22%).

3.2. Amplified SSR

In this study, from the 150 SSR loci considered in *A. ostoyae*, 105 were successfully amplified, whereas 45 failed to amplify or showed nonspecific PCR products.

3.3. SSR polymorphism

Using the 105 successfully amplified SSR loci, we analyzed 22 strains of *A. ostoyae*. The M_{AF} values ranged from 0.225 (FEL-AO-124) to 1.000 (FEL-AO-046), with an average of 0.589. Only one if the 105 loci analyzed (FEL-AO-046) was monomorphic, as indicated by $N_A = 1$, $H_E = 0$, $H_O = 0$, and PIC = 0. The remaining loci were polymorphic, with N_A ranging from 2 to 11 (FEL-AO-071) and an average of 5.4 alleles per locus. H_E ranged from 0.000 (FEL-AO-046) to 0.850 (FEL-AO-080), with an average of 0.541. H_O was 0.000 (observed in 14 loci) to 0.857 (FEL-AO-080), with an average 0.255. The PIC values ranged from 0.000 (FEL-AO-046) to 0.834 (FEL-AO-049), with an average of 0.504; 19 loci exhibited a PIC value of 0.7 or higher (Table 4). These highly polymorphic markers provide robust tools for studying genetic variation and assessing population dynamics in *Armillaria* species [44,45]. In UPGMA clustering analysis, the 22 strains were broadly divided into four groups (Figure 1).

Compared with SSR markers for the analysis of other *Armillaria* species, N_A of eight EST-based SSR markers developed from 404 *A. luteo-virens* resources from 23 wild populations ranged from 4 to 15 with an average of 8.75, H_O ranged from 0.451–0.485 with an average of 0.472, and H_E ranged from 0.513 to 0.549 with an average 0.525 and SSR markers of *A. cepistipes* from 25 resources each from the Swiss population and the Ukrainian population were as follows: N_A ranged from 1 to 8 with an average of 4.68, H_O ranged from 0.04 to 0.84 with an average of 0.478, and H_E ranged from 0.20 to 0.84 with an average of 0.601 [36]. Although a direct comparison is difficult because the resources were different, the SSR marker developed using *A. ostoyae* in this study had a lower H_O than that in the previous study.

3.4. Transferability of A. ostoyae SSR markers to A. gallica

Among the 105 SSR loci tested, 52 were successfully amplified in *A. gallica*. The transferability of 20 *Pinus koraiensis* SSR markers was assessed across 7 closely related species (45%) [46]. Of the 600 markers developed for *Myrica rubra*, 91.14% were successfully amplified in *Myrica adenophora*, while *Myrica nana* showed a success rate of 89.87%, and

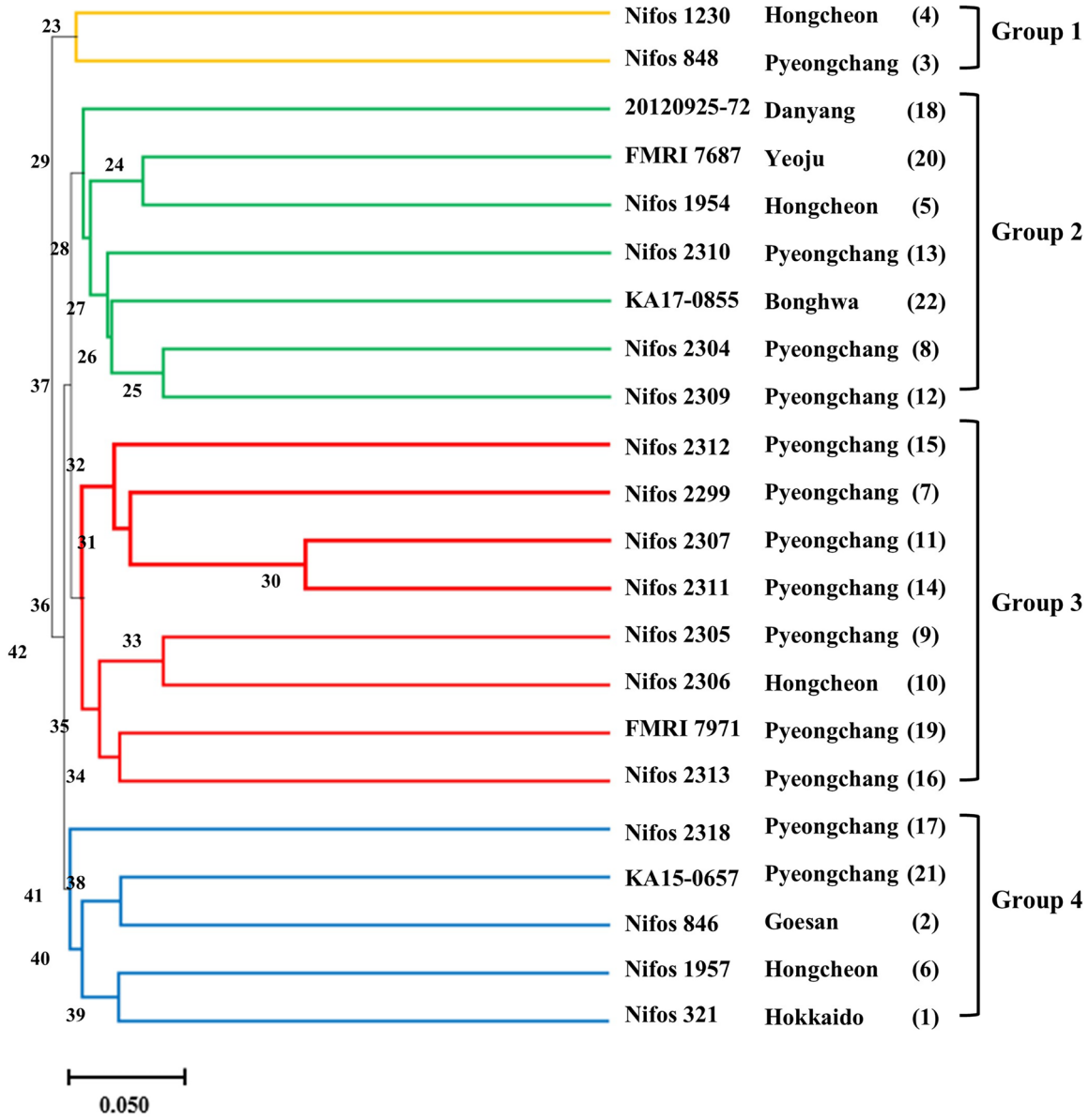

Figure 1. UPGMA dendrogram of 22 *Armillaria ostoyae* strains based on shared allele method. Numbers on the branches indicate genetic distance values, and numbers in parentheses represent strain numbers.

Myrica cerifera exhibited a transferability rate of 46.84% [47]. Similarly, among 78 EST-SSR markers developed for *Triticun aestivum*, the transferability to *Hordeum vulgare* was 55% [47]. In the genus *Armillaria*, eight SSR markers for *A. cepistipes*, six markers for *A. galiica*, four markers for *A. ostoyae*, one marker for *A. borealis*, and two markers for *A. mellea* were polymorphic, confirming the cross-species PCR amplification [36]. The results of this study further indicate the potential transferability of gSSR markers developed from *A. ostoyae* to the related *A. gallica*. Upon evaluating the diversity of amplified markers in *A. gallica*, the M_{AF} ranged from a minimum of 0.214 (FEL-AO-136) to a maximum of 1.000 (FEL-AO-102, FEL-AO-118), with a mean of 0.615. The N_A ranged from 1 (FEL-AO-102, FEL-AO-118) to 10 (FEL-AO-114), with an average of 4.3 alleles. The H_E ranged from a minimum of 0.000 (FEL-AO-102 and, FEL-AO-118) to a maximum of 0.860 (FEL-AO-136), with a mean of 0.517, while the H_O was 0.000 at 19 loci, with the highest value of 0.563 observed in FEL-AO-001. The PIC value ranged from 0.000 (FEL-AO-102, FEL-AO-118) to 0.844 (FEL-AO-136), with a mean of 0.475; six loci exhibited a PIC value of 0.7 or higher (Table 5). The successful cross-species amplification of SSR markers between *A. ostoyae* and *A. gallica* suggested a degree of genetic conservation between these species, highlighting their potential transferability to related species [48]. This observed transferability sets the stage for future research that incorporates broader genomic data to explore further the genetic structure and evolutionary dynamics of these species [48]. Furthermore, UPGMA clustering identified five distinct groups, indicating clear genetic differentiation the among individuals (Figure 2). This distinct

Table 5. Diversity statistics from selected 52 SSR markers used for analyzing 16 *Armillaria gallica* strains.

Marker	M_{AF}	N_A	H_E	H_O	PIC
FEL-AO-001	0.438	7	0.701	0.563	0.655
FEL-AO-006	0.625	4	0.555	0.000	0.510
FEL-AO-012	0.909	2	0.165	0.000	0.152
FEL-AO-013	0.750	3	0.406	0.000	0.371
FEL-AO-014	0.750	3	0.406	0.000	0.371
FEL-AO-018	0.464	4	0.620	0.214	0.546
FEL-AO-035	0.633	3	0.518	0.200	0.451
FEL-AO-042	0.600	5	0.562	0.067	0.505
FEL-AO-049	0.250	6	0.813	0.250	0.786
FEL-AO-050	0.688	2	0.430	0.000	0.337
FEL-AO-054	0.625	4	0.539	0.000	0.483
FEL-AO-057	0.563	3	0.570	0.000	0.496
FEL-AO-060	0.400	4	0.684	0.133	0.624
FEL-AO-064	0.625	4	0.525	0.063	0.459
FEL-AO-071	0.594	5	0.594	0.375	0.554
FEL-AO-073	0.893	2	0.191	0.071	0.173
FEL-AO-075	0.500	4	0.564	0.071	0.470
FEL-AO-077	0.844	3	0.271	0.063	0.248
FEL-AO-080	0.313	9	0.828	0.313	0.810
FEL-AO-082	0.750	2	0.375	0.000	0.305
FEL-AO-083	0.594	5	0.576	0.063	0.525
FEL-AO-084	0.625	6	0.578	0.000	0.553
FEL-AO-092	0.656	4	0.525	0.313	0.486
FEL-AO-093	0.344	6	0.740	0.438	0.698
FEL-AO-095	0.917	2	0.153	0.000	0.141
FEL-AO-099	0.563	4	0.578	0.000	0.510
FEL-AO-100	0.500	5	0.653	0.000	0.602
FEL-AO-102	1.000	1	0.000	0.000	0.000
FEL-AO-104	0.625	4	0.539	0.000	0.483
FEL-AO-110	0.781	5	0.375	0.125	0.357
FEL-AO-111	0.500	3	0.620	0.000	0.548
FEL-AO-112	0.594	7	0.619	0.188	0.599
FEL-AO-113	0.800	4	0.347	0.133	0.329
FEL-AO-114	0.429	10	0.770	0.429	0.752
FEL-AO-115	0.750	3	0.401	0.357	0.359
FEL-AO-118	1.000	1	0.000	0.000	0.000
FEL-AO-120	0.433	4	0.633	0.267	0.560
FEL-AO-122	0.455	6	0.711	0.273	0.673
FEL-AO-125	0.375	5	0.727	0.250	0.682
FEL-AO-127	0.594	4	0.561	0.063	0.498
FEL-AO-129	0.400	4	0.660	0.000	0.596
FEL-AO-130	0.429	7	0.737	0.357	0.705
FEL-AO-131	0.594	5	0.547	0.125	0.475
FEL-AO-132	0.467	9	0.742	0.267	0.723
FEL-AO-133	0.656	4	0.525	0.063	0.486
FEL-AO-134	0.867	3	0.240	0.000	0.227
FEL-AO-135	0.769	3	0.379	0.000	0.343
FEL-AO-136	0.214	9	0.860	0.429	0.844
FEL-AO-142	0.889	2	0.198	0.000	0.178
FEL-AO-145	0.643	5	0.551	0.143	0.521
FEL-AO-146	0.594	5	0.594	0.125	0.554
FEL-AO-148	0.733	4	0.429	0.133	0.393
MAX	1.000	10	0.860	0.563	0.844
MIN	0.214	1	0.000	0.000	0.000
Mean	0.615	4.38	0.517	0.133	0.475

M_{AF}: major allele frequency; N_A: number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; PIC: polymorphism information content.

clustering pattern demonstrates that the developed SSR markers are effective in detecting genetic diversity [49], making them valuable tools for further population genetics studies.

4. Conclusion

In the present study, we identified SSR loci in the whole genome of *A. ostoyae* and developed 150 SSR markers to evaluate *Armillaria*. Of these, 105 markers were successfully amplified and analyzed. We assessed the potential transferability of the related species, *A. gallica* and confirmed its transferability using 52 of 105 markers (49.5%).

These markers are expected to serve as valuable tools for evaluating genetic diversity, analyzing population structure, efficiently selecting traits within the *Armillaria* genus, and in other related studies. Further investigations are required to determine the transferability of these markers to other closely related species and their practical applications. Future research should focus on validating these markers across a broad range of *Armillaria* species and investigating their utility in ecosystem management, disease control, and breeding for advantageous traits.

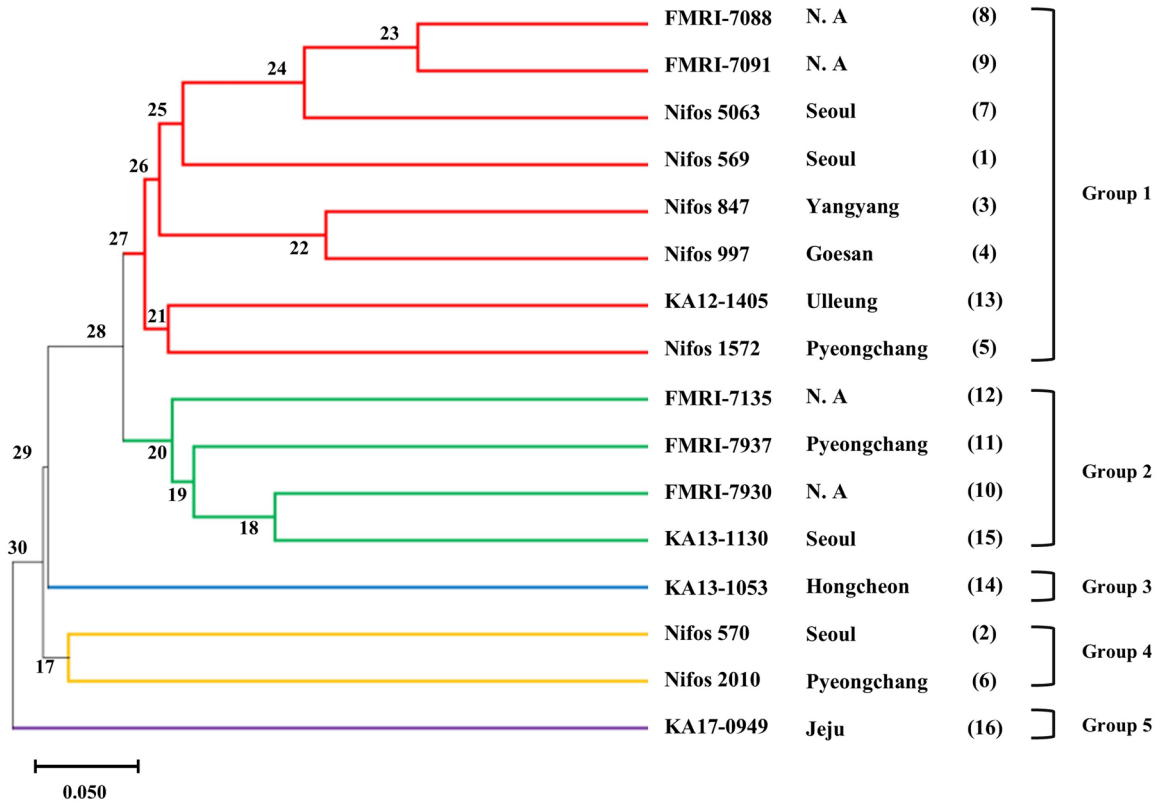

Figure 2. UPGMA dendrogram of 16 *Armillaria gallica* strains based on based on shared allele method. Numbers on the branches indicate genetic distance values, and numbers in parentheses represent strain numbers.

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Author contributions

S.K.: Conceptualization, methodology, investigation, formal analysis, visualization, and writing—original draft. H.L.: Conceptualization, data curation, supervision, and writing—original draft, reviewing, and editing. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Baumgartner K, Coetzee MP, Hoffmeister D. Secrets of the subterranean pathosystem of *Armillaria*. Mol Plant Pathol. 2011;12(6):515–534. doi: 10.1111/j.1364-3703.2010.00693.x.
[2] Volk TJ. A nomenclatural study of *Armillaria* and *Armillariella* species. Synopsis Fungorum. 1995;8:1–121.
[3] Sipos G, Prasanna AN, Walter MC, et al. Genome expansion and lineage-specific genetic innovations in the forest pathogenic fungi *Armillaria*. Nat Ecol Evol. 2017;1(12):1931–1941. doi: 10.1038/s41559-017-0347-8.
[4] Guillaumin JJ, Mohammed C, Anselmi N, et al. Geographical distribution and ecology of the *Armillaria* species in western Europe. Eur J For Pathol. 1993;23(6–7):321–341. doi: 10.1111/j.1439-0329.1993.tb00814.x.
[5] Tsykun T, Rigling D, Prospero S, et al. Diversity and ecology of *Armillaria* species in virgin forests in the Ukrainian Carpathians. Mycol Prog. 2012;11(2):403–414. doi: 10.1007/s11557-011-0755-0.
[6] Heinzelmann R, Dutech C, Tsykun T, et al. Latest advances and future perspectives in *Armillaria* research. Can J Plant Pathol. 2019;41(1):1–23. doi: 10.1080/07060661.2018.1558284.
[7] Tsykun T, Rellstab C, Dutech C, et al. Comparative assessment of SSR and SNP markers for inferring the population genetic structure of the common fungus *Armillaria cepistipes*. Heredity. 2017;119(5):371–380. doi: 10.1038/hdy.2017.48.
[8] Bendel M, Kienast F, Rigling D. Genetic population structure of three *Armillaria* species at the landscape scale: a case study from Swiss *Pinus mugo* forests. Mycol Res. 2006;110(6):705–712. doi: 10.1016/j.mycres.2006.02.002.

[9] Langrell SRH, Lung-Escarmant B, Decroocq S. Isolation and characterization of polymorphic simple sequence repeat loci in *Armillaria ostoyae*. Mol Ecol Notes. 2001;1(4):305–307. doi: 10.1046/j.1471-8278.2001.00119.x.
[10] Erbiai E, da Silva EH, Saidi LP, et al. Chemical composition, bioactive compounds, and antioxidant activity of two wild edible mushrooms *Armillaria mellea* and *Macrolepiota procera* from two countries (Morocco and Portugal). Biomolecules. 2021;11(4):575. doi: 10.3390/biom11040575.
[11] Engels B, Heinig U, Grothe T, et al. Cloning and characterization of an *Armillaria gallica* cDNA encoding protoilludene synthase, which catalyzes the first committed step in the synthesis of antimicrobial melleolides. J Biol Chem. 2011;286(9):6871–6878. doi: 10.1074/jbc.M110.165845.
[12] Kim SB, Shin HC, Lee TS, et al. Inhibitive effect of mouse sarcoma 180 by crude polysaccharide extracted from fruiting body of *Armillaria mellea*. Korean J Mycol. 2006;34(2):98–104.
[13] Yao L, Lv J, Duan C, et al. *Armillaria mellea* fermentation liquor ameliorates p-chlorophenylalanine-induced insomnia associated with the modulation of serotonergic system and gut microbiota in rats. J Food Biochem. 2022;46(2):e14075. doi: 10.1111/jfbc.14075.
[14] Lee HY, Moon S, Shim D, et al. Development of 44 novel polymorphic SSR markers for determination of shiitake mushroom (*Lentinula edodes*) cultivars. Genes. 2017;8(4):109. doi: 10.3390/genes8040109.
[15] Wang LN, Gao W, Chen Y, et al. Identification of commercial cultivars of *Agaricus bisporus* in China using genome-wide microsatellite markers. J Integr Agric. 2019;18(3):580–589. doi: 10.1016/S2095-3119(18)62126-4.
[16] Boiko SM. Identification of novel SSR markers for predicting the geographic origin of fungus *Schizophyllum commune* Fr. Fungal Biol. 2022;126(11-12):764–774. doi: 10.1016/j.funbio.2022.09.005.
[17] Franco J, Crossa J, Ribaut JM, et al. A method for combining molecular markers and phenotypic attributes for classifying plant genotypes. Theor Appl Genet. 2001;103(6-7):944–952. doi: 10.1007/s001220100641.
[18] Wu B, Hussain M, Zhang W, et al. Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. Mycology. 2019;10(3):127–140. doi: 10.1080/21501203.2019.1614106.
[19] Zane L, Bargelloni L, Patarnello T. Strategies for microsatellite isolation: a review. Mol Ecol. 2002;11(1):1–16. doi: 10.1046/j.0962-1083.2001.01418.x.
[20] Yun YE, Yu JN, Lee BY, et al. An introduction to microsatellite development and analysis. Korean J Plant Taxon. 2011;41(4):299–314. doi: 10.11110/kjpt.2011.41.4.299.
[21] Van Deynze A, Sorrells ME, Park WD, et al. Anchor probes for comparative mapping of grass genera. Theor Appl Genet. 1998;97(3):356–369. doi: 10.1007/s001220050906.
[22] Tikhonov AP, SanMiguel PJ, Nakajima Y, et al. Colinearity and its exceptions in orthologous *adh* regions of maize and sorghum. Proc Natl Acad Sci U S A. 1999;96(13):7409–7414. doi: 10.1073/pnas.96.13.7409.
[23] Kuleung C, Baenziger PS, Dweikat I. Transferability of SSR markers among wheat, rye, and triticale. Theor Appl Genet. 2004;108(6):1147–1150. doi: 10.1007/s00122-003-1532-5.
[24] Prodorutti D, Pertot I, Gessler C, et al. Genetic diversity of *Armillaria* spp. infecting highbush blueberry in northern Italy (Trentino region). Phytopathology. 2009;99(6):651–658. doi: 10.1094/PHYTO-99-6-0651.
[25] Xing R, Gao QB, Zhang FQ, et al. Genetic diversity and population structure of *Armillaria luteo-virens* (Physalacriaceae) in Qinghai-Tibet Plateau revealed by SSR markers. Biochem Syst Ecol. 2014;56:1–7. doi: 10.1016/j.bse.2014.04.006.
[26] Elías-Román RD, Medel-Ortiz R, Alvarado-Rosales D, et al. *Armillaria mexicana*, a newly described species from Mexico. Mycologia. 2018;110(2):347–360. doi: 10.1080/00275514.2017.1419031.
[27] Coetzee MPA, Wingfield BD, Wingfield MJ. *Armillaria* root-rot pathogens: species boundaries and global distribution. Pathogens. 2018;7(4):83. doi: 10.3390/pathogens7040083.
[28] Chillali M, Wipf D, Guillaumin JJ, et al. Delineation of the European *Armillaria* species based on the sequences of the internal transcribed spacer (ITS) of ribosomal DNA. New Phytol. 1998;138(3):553–561. doi: 10.1046/j.1469-8137.1998.00124.x.
[29] Park KH, Oh SY, Park MS, et al. Re-evaluation of *Armillaria* and *Desarmillaria* in South Korea based on ITS/tef1 sequences and morphological characteristics. For Pathol. 2018;48(6):e12447. doi: 10.1111/efp.12447.
[30] Ferguson BA, Dreisbach TA, Parks CG, et al. Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. Can J For Res. 2003;33(4):612–623. doi: 10.1139/x03-065.
[31] Smith ML, Bruhn JN, Anderson JB. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature. 1992;356(6368):428–431. doi: 10.1038/356428a0.
[32] Zhang T, Cai G, Rong X, et al. A combination of genome mining with an OSMAC approach facilitates the discovery of and contributions to the biosynthesis of melleolides from the Basidiomycete *Armillaria tabescens*. J Agric Food Chem. 2022;70(39):12430–12441. doi: 10.1021/acs.jafc.2c04079.
[33] Walterová L, Botella L, Hejna O, et al. Characterization of mycoviruses in *Armillaria ostoyae* and *A. cepistipes* in the Czech Republic. Viruses. 2024;16(4):610. doi: 10.3390/v16040610.
[34] Caballero JRI, Lalande BM, Hanna JW, et al. Genomic comparisons of two *Armillaria* species with different ecological behaviors and their associated soil microbial communities. Microb Ecol. 2023;85(2):708–729. doi: 10.1007/s00248-022-01989-8.
[35] Nagy S, Poczai P, Cernák I, et al. PICcalc: an online program to calculate polymorphic information content for molecular genetic studies. Biochem Genet. 2012;50(9–10):670–672. doi: 10.1007/s10528-012-9509-1.
[36] Prospero S, Jung E, Tsykun T, et al. Eight microsatellite markers for *Armillaria cepistipes* and their transferability to other *Armillaria* species. Eur J Plant Pathol. 2010;127(2):165–170. doi: 10.1007/s10658-010-9594-0.
[37] Ohm RA, de Jong JF, Lugones LG, et al. Genome sequence of the model mushroom *Schizophyllum commune*. Nat Biotechnol. 2010;28(9):957–963. doi: 10.1038/nbt.1643.

[38] Stajich JE, Wilke SK, Ahrén D, et al. Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). Proc Natl Acad Sci U S A. 2010;107(26):11889–11894. doi: 10.1073/pnas.1003391107.

[39] Martin F, Aerts A, Ahrén D, et al. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature. 2008;452(7183):88–92. doi: 10.1038/nature06556.

[40] Qu J, Huang C, Zhang J. Genome-wide functional analysis of SSR for an edible mushroom *Pleurotus ostreatus*. Gene. 2016;575(2 Pt 2):524–530. doi: 10.1016/j.gene.2015.09.027.

[41] Karaoglu H, Lee CMY, Meyer W. Survey of simple sequence repeats in completed fungal genomes. Mol Biol Evol. 2005;22(3):639–649. doi: 10.1093/molbev/msi057.

[42] Zamanmirabadi A, Hemmati R, Dolatabadian A, et al. Status of SSR, cSSR, iSSR and VNTR motifs in *Leptosphaeria maculans* based on high throughput sequencing data. Mycol Iran. 2021;8(2):95–107.

[43] Lee HY, Raveendar S, An H, et al. Development of polymorphic simple sequence repeat markers using high-throughput sequencing in button mushroom (*Agaricus bisporus*). Mycobiology. 2018;46(4):421–428. doi: 10.1080/12298093.2018.1538072.

[44] Lee JW, Lee Mw An JY, et al. Microsatellite DNA fingerprinting analysis of natural monument Gingko trees using microsatellite markers. J Korean For Soc. 2017;106(4):408–416.

[45] Cho KS, Won HS, Jeong HJ, et al. Development of multiplex microsatellite marker set for identification of Korean potato cultivars. Korean J Hortic Sci Technol. 2011;29(4):366–373.

[46] Li X, Liu X, Wei J, et al. Development and transferability of EST-SSR markers for *Pinus koraiensis* from cold-stressed transcriptome through Illumina sequencing. Genes. 2020;11(5):500. doi: 10.3390/genes11050500.

[47] Jiao Y, Jia HM, Li XW, et al. Development of simple sequence repeat (SSR) markers from a genome survey of Chinese bayberry (*Myrica rubra*). BMC Genom. 2012;13(1):201. doi: 10.1186/1471-2164-13-201.

[48] Mengistu FG, Motoike SY, Caixeta ET, et al. Cross-species amplification and characterization of new microsatellite markers for the macaw palm, *Acrocomia aculeata* (Arecaceae). Plant Genet Resour. 2016;14(3):163–172.

[49] Hur M, Lee J, Kim Y, et al. Development of whole genome sequence-based novel SSR markers in *Astragalus membranaceus* (Fisch.). Korean J Med Crop Sci. 2021;29(6):418–424. doi: 10.7783/KJMCS.2021.29.6.418.