Development of Polymorphic Simple Sequence Repeat Markers using High-Throughput Sequencing in Button Mushroom (Agaricus bisporus)

Hwa-Yong Lee, Sebastin Raveendar, Hyejin An, Youn-Lee Oh, Kab-Yeul Jang, Won-Sik Kong, Hojin Ryu, Yoon-Sup So and Jong-Wook Chung

1Department of Forest Science, Chungbuk National University, Cheongju, Republic of Korea; 2National Agrobiodiversity Center, National Institute of Agricultural Science, RDA, Jeonju, Republic of Korea; 3Department of Industrial Plant Science and Technology, Chungbuk National University, Cheongju, Republic of Korea; 4Mushroom Science Division, National Institute of Horticultural and Herbal Science, RDA, Eumseong, Republic of Korea; 5Department of Biology, Chungbuk National University, Cheongju, Republic of Korea; 6Department of Crop Science, Chungbuk National University, Cheongju, Republic of Korea

ABSTRACT

The white button mushroom (Agaricus bisporus) is one of the most widely cultivated species of edible mushroom. Despite its economic importance, relatively little is known about the genetic diversity of this species. Illumina paired-end sequencing produced 43,871,558 clean reads and 69,174 contigs were generated from five offspring. These contigs were subsequently assembled into 57,594 unigenes. The unigenes were annotated with reference genome in which 6,559 unigenes were associated with clusters, indicating orthologous genes. Gene ontology classification assigned many unigenes. Based on genome data of the five offspring, 44 polymorphic simple sequence repeat (SSR) markers were developed. The major allele frequency ranged from 0.42 to 0.92. The number of genotypes and the number of alleles ranged from 1 to 4, and from 2 to 4, respectively. The observed heterozygosity and the expected heterozygosity ranged from 0.00 to 1.00, and from 0.15 to 0.64, respectively. The polymorphic information content value ranged from 0.14 to 0.57. The genetic distances and UPGMA clustering discriminated offspring strains. The SSR markers developed in this study can be applied in polymorphism analyses of button mushroom and for cultivar discrimination.

1. Introduction

The white button mushroom (Agaricus bisporus) belongs to the genus Agaricus, family Agaricaceae, order Agaricales [1]. It is a plant biomass degrading fungus with a wide geographical distribution [2]. Apart from its ecological role, A. bisporus is considered as a healthy functional food due to the high contents of polyphenols, vitamins, minerals, polysaccharides, and proteins [3]. The button mushroom is one of the most widely cultivated edible mushrooms in the world and is the basis of a multibillion dollar industry [4,5]. Its cultivation began in the early 17th century in France [6], however, since 1950s, the production of this mushroom has been continued to increase worldwide and now represents 30% of the global mushroom production [7]. Currently, North America, Europe, India, and China are major mushroom cultivating regions [8], and cultivation efforts have recently increased in China and Korea [9]. Developing new cultivars is essential, as the mushroom production is limited owing to their complex life cycle patterns.

A. bisporus has a bipolar mating system [10], which can vary depending on varieties [4]. The A. bisporus var. burnetii and A. bisporus var. eurotetrusporus varieties produce spores with single nucleus. This enables breeding of new cultivars through conventional mating. The wild varieties have been used to generate commercial lines. However, A. bisporus var. bisporus generally shows better quality than A. bisporus var. burnetii in the commercial lines [11]. The cultivation of the button mushroom is dominated by a single hybrid strain produced from cross breeding of the Horst U1 and U3 strains developed early 1980s in the Netherlands [12]. Currently, most hybrid varieties are genetically similar to Horst U1, which has resulted in very low diversity. This lack of diversity makes it difficult to discriminate between cultivars.

Many countries have signed the treaty with the International Union for the Protection of New Varieties of Plants and have implemented laws related to breeders’ rights [13]. Moreover, the demand for royalties on the use of cultivated...
varieties is also expected to increase [14]. Therefore, there is an urgent need to develop a precise discrimination method for cultivars for the protection of breeder’s rights.

The morphological and biochemical characteristics of plants are the main basis of cultivar discrimination [15]. Similarly, in mushrooms, morphological characteristics that include color, shape, size, attachment, ornamentation, and other developmental process of fruiting bodies have been used [16]. However, these methods have limitations in their ability to discriminate closely-related cultivars, especially concerning environmentally sensitive traits [17]. The use of DNA-based molecular markers could overcome this problem, which makes it possible to evaluate genetic characters more easily and accurately than using morphological characters [18]. In this regard, molecular techniques that include restriction fragment length polymorphism (RFLP), randomly amplified polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP), single nucleotide polymorphism (SNP), and simple sequence repeat (SSR) have been widely used in the field of breeding for the evaluation of genetic diversity, introduction and selection of genetic resources, and discrimination of varieties [19–23].

Among the molecular markers, SSR microsatellite markers can be used to identify polymorphisms due to differences in the frequency of repetition in a genome. Moreover, SSR markers are highly polymorphic and thus have been widely used to evaluate genetic diversity and relationships [24]. SSR markers can be developed from genomic or expressed sequence tag (EST) libraries [25]. However, the genomic SSRs distributed throughout the genome have a great advantage over EST SSRs, which are only distributed in the transcribed region [26].

The present study aimed to develop genomic SSR markers to discriminate parental strains of button mushroom cultivars, which may provide a sound basis for button mushroom breeding programs.

2. Materials and methods

2.1. Strains and sample collection

Six strains of A. bisporus, including S1038-211, S1346-15, S1346-17, S1346-20, S1346-26, and S1346-110, were obtained from the National Institute of Horticultural and Herbal Science (http://www.nihhs.go.kr/). These strains originated from two parental strains, ASI 1038 and ASI 1346, which have been frequently used for the development of new cultivars in Korea [27]. The mycelia of each strain were cultured on CDA medium in the dark at 25 °C for a month.

2.2. DNA extraction and sequencing

The extraction of genomic DNA from the cultured mycelia was performed using a GenEX Plant Kit (GeneAll Biotechnology Co. Ltd., Seoul, Korea) following the manufacturer’s instructions. The extracted genomic DNA was subjected to high-throughput methods of re-sequencing. Illumina paired-end DNA library (average insert size of 500 bp) was constructed using the Illumina TruSeq library preparation kit following the manufacturer’s instructions. The libraries were sequenced with 2 × 300 bp on the Illumina MiSeq platform at LabGenomics (http://www.Lab.genomics.com/kor/).

2.3. Genome assembly

The genomic reads generated by the Illumina MiSeq platform were quality-trimmed with Trimmomatic ver. 0.33 to obtain reads with high quality (Phred score >20). These were de novo assembled into contigs using Platanus ver. 1.2.1 with default parameters [28]. Scaffolds were generated from contigs using Platanus ver. 1.2.1 with default parameters. Those exceeding 500 bp were selected for further study. De novo assemblies of five samples were performed independently to generate contigs and scaffolds sets for each sample.

2.4. Gene prediction and functional annotation

Genes in scaffold sequences were predicted based on a self-training algorithm using GeneMark-ES ver 4.32 [29]. Deduced protein sequences of five samples were compared with each other based on the similarity with protein sequences from a reference genome, A. bisporus (GenBank Accession No: GCF_000300575.1). BLASTP searches with cut-off E-value of 1e−4 were employed to investigate similarity. Protein sequences specific or common to each of five samples were searched and depicted by a Venn diagram. Five gene sets of five samples and the reference genome were compared using BLAST ver. 2.3.31 and then specific genes were selected for gene ontology (GO) analysis using Blast2GO [30]. GO terms assigned to three categories—Molecular function, Biological process, and Cellular component—were investigated and the frequency of GO terms was compared among the five gene sets.

2.5. SSR identification and primer design

MiCroSAtellite software was used as the identification tool to detect the SSR markers present in the cp genome (http://pgrc.ipk-gatersleben.de/misa/). This software allows the localization and identification of both perfect and compound microsatellites.
with 1 to 6 nucleotides in the basic repeat unit. We selected the 1,858 SSR motif, which showed the largest diversity among all SSR motifs, and selected a total of 91 SSR markers. Primer design parameters were set as follows: length range, 18–23 nucleotides with 21 as the optimum; PCR product size range, 200–300 bp; optimum annealing temperature (Ta), 55°C; and GC content 50–60%, with 51% as the optimum.

### 2.6. Validation of polymorphic SSR markers

The selected SSR markers were used for the identification of genetic diversity in the six *A. bisporus* strains. The DNA extracted from these strains for PCR templates was quantified with a model K5600 micro-spectrophotometer (Shanghai Biotechnology Co. Ltd., Shanghai, China) and diluted to 20 ng/μL. PCR was performed with 20 ng of template DNA using 2× PCR Master Mix Solution (i-Taq, iNtRON Biotechnology Inc., Seongnam, Korea) according to the manufacturer’s recommendations. Final volume and concentration of primers in all reactions were fixed to 20 μL and 10 μM, respectively. PCR reactions were performed as follows: 95°C for 3 min; 35 cycles of 95°C for 30 s, 55°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 fragment analyzer (Advanced Analytical Technologies Inc., Ankeny, IA, USA). Allele size was scored with PRO Size® 2.0 software (Advanced Analytical Technologies Inc.). To calculate the genetic diversity of each SSR locus, the major allele frequency (MAF), number of genotypes (NG), number of alleles (NA), observed heterozygosity (HO), expected heterozygosity (HE), and polymorphic information content (PIC) values were calculated with Power Marker V3.25. The genetic distance between the strains were calculated by the Shared Allele method and a dendrogram was constructed using the unweighted pair-group mean algorithm (UPGMA) method.

### 3. Results and discussion

Five different *Agaricus* offspring were sequenced with the Illumina MiSeq system, which produced 6,358,118–9,337,246 paired-end raw reads. A general summary of the statistical data on sequencing and assembly is provided in Table 1. The genomic reads were quality-trimmed with Trimmomatic ver. 0.33. The total number of valid reads after quality trimming (Phred score >20) was 5,980,422–8,764,486. Then the quality-trimmed reads were de novo assembled separately using Platanus ver. 1.2.1, which produced 10,676–11,841 completely assembled contigs. The overall sequence assembly produced 2,497–3,614 scaffolds ensuring the rules of minimum length requirement (500 bp).

In total, 9,323–9,875 gene models were predicted, with average size of 1,394–1,448 bp among the five samples with GeneMark-ES ver 4.32 software program (Table 1). The deduced protein sequences were compared with the *A. bisporus* reference genome. The BLASTP searches with a cut-off E-value of 1e-4 indicated that the annotated genes were highly homologous with *A. bisporus* in the database. The Venn diagram (Figure 1) illustrated the interrelation of Blast hits for predicted unigenes against the *A. bisporus* reference genome database. The results revealed that 6,559 unigenes could be considered as orthologous genes across all five samples, as shown in the Venn diagram.

To obtain better understanding on the functions of the predicted unigenes, GO classification was applied (Figure 2). The GO classifications with the reference genome assigned many unigenes to a wide range of GO categories. Different numbers of annotated unigenes were distributed under different GO categories, with 1,554 unigenes for biological

### Table 1. Summary of Illumina paired-end sequencing and assembly for *A. bisporus* used in this study.

| Sample       | Raw Reads | Cleaned Reads | Contigs | N50 | Scaffolds | N50 | Predicted Gene | Avg Gene Length |
|--------------|-----------|---------------|---------|-----|-----------|-----|----------------|-----------------|
| S1038-211    | 7,338,790 | 7,080,774     | 11,414  | 5,019| 3,226     | 30,120| 9875           | 1394            |
| S1346-110    | 9,337,246 | 8,764,486     | 11,268  | 5,306| 2,681     | 42,867| 9627           | 1448            |
| S1346-15     | 6,440,746 | 6,089,334     | 11,841  | 4,112| 3,316     | 25,673| 9518           | 1395            |
| S1346-17     | 6,358,118 | 5,980,422     | 12,374  | 3,739| 3,614     | 20,952| 9323           | 1395            |
| S1346-20     | 8,107,872 | 7,620,128     | 10,676  | 5,413| 2,497     | 49,431| 9631           | 1446            |
| S1346-26     | 8,909,410 | 8,336,414     | 11,601  | 4,811| 2,803     | 38,055| 9620           | 1433            |
processes, 722 unigenes for cellular components, and 454 unigenes for molecular functions, which indicated the high accuracy of the annotation. However, GO classification with five samples revealed only 221–552 unigenes related to different GO classes, which was markedly less compared to the reference genome. The GO annotation analyses revealed 90–302 unigenes for biological processes, 54–174 unigenes for cellular components and 20–76 unigenes for molecular functions, which indicated the reduced number of unigene annotations.

The frequency distribution of the SSRs depending on the repeat type is shown in Figure 3. According to the motif type, the SSR motifs included 3 bp with 898 (48%), 2 bp with 527 (28%), 4 bp with 221 (12%), 6 bp with 123 (7%) and 5 bp with 89 (5%). Among the 91 selected SSR markers, 43 were polymorphic and the major allele frequency (MAF) ranged from 0.42–0.92, with an average of 0.711. The number of genotypes (NG) ranged from 1–4 with an average of 2.3, whereas the number of alleles (NA) ranged from 2–4 with an average of 2.5. The observed heterozygosity (HO), expected heterozygosity (HE), and polymorphic information content (PIC) averaged 0.291, 0.395, and 0.340, respectively (Table 2). The UPGMA clustering showed that the six button mushroom strains could be divided into two clusters when analyzed with the 43 SSR markers. All offspring could be discriminated from the parental cultivars (Figure 4).
**Table 2.** Diversity statistics of the 43 polymorphic simple sequence repeats (SSR) markers in *A. bisporus* used in this study.

| Locus | SSR motif | Primer Sequence (5'-3') | M_{OE} | N_{O} | N_{A} | H_{O} | H_{E} | PIC |
|-------|-----------|-------------------------|--------|------|------|-------|-------|-----|
| AB-gSSR-0113 | (AG) | F-TACTCAACACAGATGCTGATTG | 0.58 | 3 | 3 | 0.5 | 0.57 | 0.5 |
| AB-gSSR-0126 | (CT) | R-CTACACGCTGCTGTTGCTCG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0139 | (AG) | F-GAATACGTAAGCTGACCTCCTG | 0.67 | 3 | 3 | 0 | 0.5 | 0.45 |
| AB-gSSR-0150 | (CT) | R-TCTAAACAGCGTCTAACAGCCT | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0179 | (CT) | F-TGAAATCGATTAGTCCTTCAACG | 0.5 | 3 | 4 | 0.83 | 0.63 | 0.56 |
| AB-gSSR-0182 | (CT) | R-CTGATTGTGTATTTCTCGTTG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0193 | (CT) | F-GAACTCTGTTAATGTTGATTG | 0.58 | 3 | 2 | 0.5 | 0.49 | 0.37 |
| AB-gSSR-0199 | (AG) | R-AGGACGACTTCGATGAGTTAAA | 0.42 | 2 | 4 | 1 | 0.64 | 0.57 |
| AB-gSSR-0218 | (AC) | F-TAATTTAATCAGCGTCTGCACT | 0.67 | 2 | 2 | 0 | 0.44 | 0.35 |
| AB-gSSR-0222 | (AC) | R-ATGTTTGAGGGCATGAGGATG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0270 | (TA) | F-AACCTCAACTGGTTAGTCGTC | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0296 | (TA) | R-ACCAACGTATCTGCTGCTGAC | 0.28 | 3 | 4 | 1 | 0.63 | 0.56 |
| AB-gSSR-0298 | (TA) | F-TTAGGTTAATGTTGATTG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0305 | (TA) | R-CAGTGTTGTTTATTCCTTCG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0391 | (TC) | F-GAATACGTAAGCTGACCTCCTG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0396 | (TA) | R-ATGTTTGAGGGCATGAGGATG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0428 | (TC) | F-AAGCTTTACACCCATTACTCATT | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0432 | (TC) | R-CAGTGTTGTTTATTCCTTCG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0444 | (TA) | F-AGCAGCTGTTAATGTTGATTG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0488 | (TC) | R-CAGTGTTGTTTATTCCTTCG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0489 | (GA) | F-CAATTTATCAGTGATGATG | 0.5 | 1 | 2 | 1 | 0.5 | 0.38 |
| AB-gSSR-0512 | (TA) | R-CAGTGTTGTTTATTCCTTCG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0562 | (CTT) | F-ACACGTATCCGAGTAGGAGA | 0.82 | 2 | 2 | 0.17 | 0.15 | 0.14 |
| AB-gSSR-0564 | (CTT) | R-ACCAACGTATCTGCTGCTGAC | 0.28 | 3 | 4 | 1 | 0.63 | 0.56 |
| AB-gSSR-0574 | (AAG) | F-GAGGAAAGTTGCTGGTAGG | 0.5 | 2 | 3 | 1 | 0.67 | 0.54 |
| AB-gSSR-0580 | (GTT) | R-ACCAACGTATCTGCTGCTGAC | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0582 | (GTT) | F-GAATACGTAAGCTGACCTCCTG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0584 | (GTT) | R-CAGTGTTGTTTATTCCTTCG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0586 | (GTT) | F-GAATACGTAAGCTGACCTCCTG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0574 | (AAG) | F-ACACGTATCCGAGTAGGAGA | 0.82 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0580 | (GTT) | R-ACCAACGTATCTGCTGCTGAC | 0.75 | 3 | 3 | 0.5 | 0.38 | 0.3 |
| AB-gSSR-0582 | (GTT) | F-GAATACGTAAGCTGACCTCCTG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0584 | (GTT) | R-CAGTGTTGTTTATTCCTTCG | 0.83 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0603 | (GTT) | F-GAATACGTAAGCTGACCTCCTG | 0.67 | 3 | 4 | 1 | 0.5 | 0.38 |
| AB-gSSR-0608 | (GTT) | R-ACCAACGTATCTGCTGCTGAC | 0.82 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0611 | (AG) | F-GAATACGTAAGCTGACCTCCTG | 0.92 | 2 | 2 | 0 | 0.28 | 0.24 |
| AB-gSSR-0630 | (CAT) | R-TACTTTACGCTATGTCGTTG | 0.5 | 3 | 3 | 0 | 0.61 | 0.54 |
| AB-gSSR-0687 | (GAT) | F-ATTAGTCACGCTATGTCGTTG | 0.58 | 2 | 2 | 0.83 | 0.49 | 0.37 |
| AB-gSSR-0709 | (TCT) | R-ATTTCTGACGCTATGTCGTTG | 0.42 | 2 | 4 | 1 | 0.64 | 0.57 |
| AB-gSSR-0713 | (AGA) | F-ATTTTGTTACACCCATTACTCATT | 0.5 | 2 | 3 | 1 | 0.57 | 0.48 |

(continued)
**A. bisporus** is one of the most cultivated edible mushrooms globally and remains an important component of the human diet. Different trials have been performed to breed the cultivars and have succeeded in the release of the first hybrid strains [31]. However, genetic manipulation by mating *A. bisporus* strains was limited due to the formation of secondary homothallic mushroom species during bisporic production of basidiospores [32]. Therefore, it is necessary to explore the correlation between the genotypes to effectively operate breeding programs. In this study, we chose five different diploid strains for genome sequencing because their genetic relationships with other strains are very limited. The genome sequences of diploid spores were analyzed using the Illumina Miseq platform. The sequence assembly produced 2,497–3,614 scaffolds (>500 bp) from the 6,358,118–9,337,246 paired-end raw reads, which revealed only partial genome assembly. In general, the high percentage of repetitive sequences and high expansion of retro-transposon gene families are considered to be major obstacles in genome assembly of mushroom species [33]. However, the next generation sequencing technologies have efficiently solved this high repeat ratio problem. The GeneMark-ES program based on a self-training algorithm predicted 9,323–9,875 gene models with average size of 1,394–1,448 bp among the five samples. In addition, 6,559 unigenes were considered as orthologous genes across all five samples, as shown by Venn diagramming. Different number of unigenes in the *Leucocalocybe mongolica* genome have been against three different databases [34]. Similarly, in the GO classification, only 221–552 unigenes were classified to predicted functions. Identification and development of genomic SSR loci using whole genomes have been successfully applied in several plants [35,36] and the mushroom [37]. In general, the wild type has higher genetic diversity than cultivars [38–41]. Therefore, the SSR markers developed using offspring in this study will reveal more diversity when applied to the wild types. The SSR markers successfully discriminated offspring from common parent and thus could be used in cultivar discrimination, molecular breeding, and studies of the genetic structure of the button mushroom.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Golden Seed

**Table 2.** Continued.

| Locus         | SSR motif | Primer Sequence (5’-3’) | *M*<sub>A</sub> | *N*<sub>G</sub> | *N*<sub>A</sub> | *H*<sub>O</sub> | *H*<sub>E</sub> | PIC  |
|---------------|-----------|-------------------------|----------------|----------------|----------------|----------------|----------------|------|
| AB-gSSR-0730  | (TCT)₄   | R-CTGGAAGACTGTTATCAGAGGG F-AGAGATGAGGGAGAATAGAGG R-AACAGGACCTCAAACACAAAGC | 0.5            | 4              | 3              | 0.33           | 0.61           | 0.54 |
| AB-gSSR-0736  | (AGT)₆   | F-CATTAGCAGTTGAGAGATGC R-ACCACTACTACTCCACGCACAC | 0.67           | 3              | 3              | 0              | 0.5            | 0.45 |
| AB-gSSR-0784  | (GCT)₅   | F-ACTCTCAACAGTCAAAGAGAGA R-CAGGACATTCACTGTTTCAC | 0.67           | 3              | 3              | 0              | 0.5            | 0.45 |
| AB-gSSR-0788  | (AGC)₃   | F-CTGGAGAATAGGAGGGAGGT R-TCCAACAATCTGCTTCCATA | 0.92           | 2              | 2              | 0.17           | 0.15           | 0.14 |
| Mean          |           |                         | 0.71           | 2.3            | 2.5            | 0.29           | 0.395          | 0.34 |

*M*<sub>A</sub>: major allele frequency; *N*<sub>G</sub>: number of genotypes; *N*<sub>A</sub>: number of alleles; *H*<sub>O</sub>: observed heterozygosity; *H*<sub>E</sub>: expected heterozygosity; PIC: Mean polymorphic information content.
Project, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) [213007-05-2-SB]36.

References

[1] Imbach EJ. Mushroom of the canton of Lucerne and neighboring central Switzerland. Mitt Naturforsch Ges Luzern. 1946;15:1–85.

[2] Morin E, Kohler A, Baker AR, et al. Genome sequence of the button mushroom Agaricus bisporus reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc Natl Acad Sci U S A. 2012;109:17501–17506.

[3] Liu J, Jia L, Kan J, et al. In vitro and in vivo antioxidative activity of ethanolic extract of white button mushroom (Agaricus bisporus). Food Chem Toxicol. 2013;51:310–316.

[4] Foulongne-Oriol M, Spataro C, Savoie JM. Novel microsatellite markers suitable for genetic studies in the white button mushroom Agaricus bisporus. Appl Microbiol Biotecnol. 2009;84:1125–1135.

[5] McGee CF. Microbial ecology of the Agaricus bisporus mushroom cropping process. Appl Microbiol Biotecnol. 2018;102:1075–1083.

[6] Savoie J-M, Mata G. Growing Agaricus bisporus as a contribution to sustainable agricultural development. In: Petre M, editor. Mushroom Biotechnology. San Diego: Academic Press; 2016. p. 69–91.

[7] Royse DJ. A global perspective on the high five: Agaricus, Pleurotus, Lentinula, Auricularia & Flammulina. In: Singh M, editor. Proceedings of the 6th International Conference on Mushroom Biology and Mushroom Products; 2011 Oct 04–07; Arcachon: INRA; 2011. p. 7–15.

[8] Kabel MA, Jurak E, Makela MR, et al. Occurrence and function of enzymes for lignocellulose degradation in commercial Agaricus bisporus cultivation. Appl Microbiol Biotecnol. 2017;101:4363–4369.

[9] Sonnenberg ASM, Baars JJP, Hendrickx PM, et al. Breeding and strain protection in the button mushroom Agaricus bisporus. In: Savoie JM, Foulongne-Oriol M, Largeteau M et al. editors. Proceedings of the 7th International Conference of the World Society for Mushroom Biology and Mushroom Products; 2011 Oct 04–07; Arcachon: INRA; 2011. p. 7–15.

[10] Raper CA, Raper JR, Miller RE. Genetic analysis of the life cycle of Agaricus bisporus. Mycologia. 1972;64:1088–1117.

[11] Gao W, Weijn A, Baars J, et al. Quantitative trait locus mapping for bruising sensitivity and cap color of Agaricus bisporus (button mushrooms). Fungal Genet Biol. 2015;77:69–81.

[12] Savoie J-M, Foulongne-Oriol M, Barroso G, et al. Genetics and genomics of cultivated mushrooms, application to breeding of agricons. In: Kempen F, editor. Agricultural Applications. Berlin: Springer-Verlag Berlin Heidelberg; 2013. p. 3–33.

[13] Sonnenberg ASM. Baars JJP, Kerrigan RW. Mushroom breeding: hurdles and challenges. In: Lelly JI, Buswell JA, editors. Proceeding of the 6th International Conference of the World Society for Mushroom Biology and Mushroom Products; 2008 Sep 29–Oct 3; Bonn: GAMU; 2008. p. 96–103.

[14] Min KJ, Kim IK, Kwak AM, et al. Genetic diversity of Agaricus bisporus strains by PCR polymorphism. Korean J Mycol. 2014;42:1–8. Korean.

[15] Hong WJ, Khaing AA, Park YJ, Cultivar identification of Chrysanthemum (Dendranthema grandiflorum. Ramat.) using SSR markers. Korean J Int Agri. 2013;25:385–394. Korean.

[16] Paisey EC, Abbas B. Morphological characteristics and nutritional values of wild types of sago mushrooms (Volvariea spp.) that growth naturally in Manokwari, West Papua. Natural Sci. 2015;7:559–604.

[17] Kauzerud H, Heggard B, Buntege U, et al. Warming-induced shift in European mushroom fruiting phenology. Proc Natl Acad Sci U S A. 2012;109:14488–14493.

[18] Szendro T, Aspelrud A, Lomnes A, et al. DNA amplification polymorphisms of the cultivated mushroom Agaricus bisporus. Appl Environ Microbiol. 1992;58:2971–2977.

[19] Iwao S, Masahiro T, Yoshio N. Discrimination of mushrooms in genus Pleurotus by DNA restriction fragment length polymorphism. J Gen Appl Microbiol. 1992;38:597–603.

[20] Mukhopadhyay K, Haque I, Bandopadhyay R, et al. AFLP based assessment of genetic relationships among shiitake (Lentinula spp.) mushrooms. Mol Biol Rep. 2012;39:6059–6065.

[21] Wang R, Becker E, Wach M. DNA amplification polymorphisms of the cultivated mushroom Agaricus bisporus. Appl Environ Microbiol. 1992;58:2971–2977.

[22] Im CH, Kim KH, Je HJ, et al. Multiple simple sequence repeat (SSR) markers discriminating Pleurotus eryngii cultivar. Korean J Mycol. 2014;42:159–164. Korean.

[23] Li YC, Korol AB, Fahima T, et al. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Mol Ecol. 2002;11:2453–2465.

[24] Thiel T, Michalek W, Varshney R, et al. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (Hordeum vulgare L.). Theor Appl Genet. 2003;106:411–422.

[25] Nam KY. Genetic polymorphism and morphological traits of collected Agaricus bisporus strains [master’s thesis]. Cheongiu: Chungbuk National University; 2018.

[26] Kajitani R, Toshimoto K, Noguchi H, et al. Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res. 2014;24:1384–1395.

[27] Lommard S, Mirouane S, Noguchi H, et al. Gene identification in novel eukaryotic genomes by self-training algorithm. Nucleic Acids Res. 2005;33:6494–6506.
[30] Götz S, García-Gómez JM, Terol J, et al. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. 2008; 36:3420–3435.

[31] Fritsche G. Breeding mushrooms. Mushroom J. 1986;157:4–17.

[32] Kerrigan RW, Royer JC, Baller LM, et al. Meiotic behavior and linkage relationships in the secondarily homothallic fungus Agaricus bisporus. Genetics. 1993;133:225–236.

[33] Stajich JE, Wilke SK, Ahren 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;107:11889–11894.

[34] Lu T, Bau T. De novo assembly and characterization of the transcriptome of a wild edible mushroom Leucocolocybe mongolica and identification of SSR markers. Biotechnol Biotechnological Equip. 2017;31:1148–1159.

[35] Cavagnaro PF, Senalik DA, Yang L, et al. Genome-wide characterization of simple sequence repeats in cucumber (Cucumis sativus L.). BMC Genomics. 2010;11:569.

[36] Wang Q, Fang L, Chen J, et al. Genome-wide mining, characterization, and development of microsatellite markers in gossypium species. Sci Rep. 2015;5:10638.

[37] Lee H-Y, Moon S, Shim D, et al. Development of 44 novel polymorphic SSR markers for determination of shiitake mushroom (Lentinula edodes) cultivars. Genes. 2017;8:109.

[38] Wang M, Li RZ, Yang WM, et al. Assessing the genetic diversity of cultivars and wild soybeans using SSR markers. Afr J Biotechnol. 2010;9:4857–4866.

[39] Ram SG, Thiruvengadam V, Vinod KK. Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. J Appl Genet. 2007;48:337–345.

[40] Liu XB, Li J, Yang ZL. Genetic diversity and structure of core collection of winter mushroom (Flammulina velutipes) developed by genomic SSR markers. Hereditas. 2018;155:3.

[41] Rokni N, Goltapeh EM, Shafeinia A, et al. Evaluation of genetic diversity among some commercial cultivars and Iranian wild strains of Agaricus bisporus by microsatellite markers. Botany. 2016;94:9–13.