# Analysis of the Genetic Diversity and Relationship within *Hypsizygus marmoreus* Based on Sequence - Related Amplification Polymorphism

**Chengshu Qiu[1-2], Wenjuan Yan[2], Bin Song[2], Peng Li[1-2], Wangqiu Deng[2] and Taihui Li[1-2]***

[1]School of Bioscience & Bioengineering, South China University of Technology, Guangzhou - 510 006, China,
[2]Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Open Laboratory of Applied Microbiology, State Key Laboratory of Applied Microbiology (Ministry—Guangdong Province Jointly Breeding Base) South China, Guangdong Institute of Microbiology, Guangzhou - 510 070, China.

(Received: 24 September 2012; accepted: 08 November 2012)

**Germplasm resources of *Hypsizygus marmoreus* were collected from different parts of China. Sequence-related amplified polymorphism (SRAP) markers were employed to evaluate the genetic diversity and relationship among the 32 collected strains of *H. marmoreus*. Fifty-five SRAP primer combinations were used and 533 polymorphic bands were observed. Phylogenetic analysis by Unweighted Pair Group Method with Arithmetic Mean based on similarity matrices indicated three clusters, namely, A, B, and C. The Jaccard's similarity coefficients of genetic similarity among all accessions ranged from 0.40 to 0.73, and averaged 0.50 among these strains collected in China, revealing a medium level of genetic diversity. The results demonstrated that SRAP can be used to evaluate the genetic diversity of *H. marmoreus* in China and to distinguish the different storage and cultivar strains. These applications are helpful for the effective evaluation, management, utilization, and conservation of germplasm resources.**

**Key words**: Germplasm source, evaluate, Unweighted Pair Group Method with Arithmetic Mean (UPGMA), China.

*Hypsizygus marmoreus* (Peck) Bigelow is an edible fungus that was successfully domesticated in Japan, and widely cultivated and commercially available in East Asia[1]. In recent years, *H. marmoreus* has become a popular mushroom because of its delicious taste and good nutrition value[2]. *H. marmoreus* has numerous hygiene and medicinal functions such as antioxidant, antitumor, antifungal, and blood pressure-lowering activities, resulting in its increasing popularity, especially in East Asia [1, 3-7].

* To whom all correspondence should be addressed. Tel.:+86-020-87137619; E-mail: mycolab@263.net

Sequence-related amplified polymorphism (SRAP) was introduced by Li and Guiros[8]. It is based on two primers that amplify open reading frames (OFRs). Compared with inter simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP), SRAP technology is more concordant with morphological diversity, more reproducible, more stable, and simpler[9, 10]. It is widely applied in the study of genetic diversity, genetic linkage map construction, species identification, and so on [8, 11, 12]. SRAP technology is also used to study the genetic diversities of edible fungi such as *Lentinula edodes*[13], *Auricularia polytricha*[14], and *Ganoderma*[15]. RAPD and AFLP markers have been used to study the

diversity of *H. marmoreus*, and SCAR markers to identify commercial and wild strains[16-18]. However, the results of previous studies could not comprehensively reflect the genetic diversity and characteristics of *H. marmoreus* due to the few primers and limited strains used in the tests. To evaluate the genetic diversity of *H. marmoreus* germplasm, 55 primer combinations of SRAP were used to investigate 32 strains collected from different parts of China. Two indexes of SRAP markers were computed to distinguish the strains in this study.

## MATERIALS AND METHODS

### Fungus materials

Thirty-two strains of *H. marmoreus*, including 10 storage and 22 commercial cultivar strains, were collected and further verified. They were obtained from the Agricultural Culture Collection of China (ACCC), from the Provincial Academy of Agricultural Sciences, and from several institutes dealing with edible fungi and mushroom production (Table 1). Mycelia were inoculated in

**Table 1.** *Hypsizygus marmoreus* strains involved in the study

| No. | Strain code | Origin | Strain type |
|---|---|---|---|
| 1 | ACCC50474 | Dalian University of Technology, Dalian, Liaoning, China | storage strain |
| 2 | ACCC51149 | Xin Yu Edible Fungi Institute, Wuhan, Hubei, China | storage strain |
| 3 | ACCC51532 | Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China | storage strain |
| 4 | ACCC51533 | Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China | storage strain |
| 5 | ACCC51536 | Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China | storage strain |
| 6 | ACCC51583 | Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China | storage strain |
| 7 | ACCC51661 | Huan Yu Edible Fungi Institute, Jiayu, Hubei, China | storage strain |
| 8 | ACCC51729 | Nanjing Agricultural University , Nanjing, Jiangsu, China | storage strain |
| 9. | ACCC51800 | Shandong Agricultural University, Taian, Shandong, China | storage strain |
| 10 | GDGM26168 | Shanghai Finc Bio-tech Inc., Shanghai, China | commercial cultivar |
| 11 | GDGM26169 | Shandong Ronfun Group, Ltd., Dongying, Shandong, China | commercial cultivar |
| 12 | GDGM26170 | Shanghai Finc Bio-tech Inc., Shanghai, China | commercial cultivar |
| 13 | GDGM26171 | Shanghai Tianchu Mushroom Co., Ltd., Shanghai, China | commercial cultivar |
| 14 | GDGM26172 | Yingliang Agricultural Co., Ltd., Beijing, China | commercial cultivar |
| 15 | GDGM26713 | Starway Bio-technology, Dongguan, Guangdong, China | commercial cultivar |
| 16 | FJNK-1 | Fujian Academy of Agricultural Sciences, Fuzhou, China | commercial cultivar |
| 17 | FJNK-2 | Fujian Academy of Agricultural Sciences, Fuzhou, China | commercial cultivar |
| 18 | JSGY-1 | Gaoyou Institute of Edible Fungi, Gaoyou, Jiangsu, China | commercial cultivar |
| 19 | JSGY-2 | Gaoyou Institute of Edible Fungi, Gaoyou, Jiangsu, China | commercial cultivar |
| 20 | HN-1 | Hunan Academy of Agricultural Science, Changsha, Hunan, China | commercial cultivar |
| 21 | HN-2 | Hunan Academy of Agricultural Science, Changsha, Hunan, China | commercial cultivar |
| 22 | HZND-1 | Huazhong Agricultural University, Wuhan, Hubei, China | commercial cultivar |
| 23 | HBYC | Yichang Kanghuiyuan Biology Technology Co., Ltd., Yichang, Hubei, China | commercial cultivar |
| 24 | JSTD | Jiangsu Jiangdu Tianda Fungi Institute, Jiangdu, Jiangsu, China | commercial cultivar |
| 25 | ZJNKYY | Horticulture Institute of Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China | commercial cultivar |
| 26 | HNNK | Henan Academy of Agricultural Science, Zhengzhou, Henan, China | commercial cultivar |
| 27 | CYQH | Liaoning Quanhe Fungi Co., Ltd., Chaoyang, Liaoning, China | commercial cultivar |
| 28 | SM-4 | Sanming Mycological Institute, Sanming, Fujian, China | commercial cultivar |
| 29 | MYSYB | Mianyang Edible Fungi Research Institute of Sichuan, Mianyang, Sichuan, China | commercial cultivar |
| 30 | SCNK | Sichuan Academy of Agricultural Science, Chengdu, Sichuan, China | commercial cultivar |
| 31 | CBM | Changbaishan Mountain, Jilin, China | storage stain |
| 32 | SJZCX | Hebei Shijiazhuang Chuangxin Institute of Edible Fungi, Shijiazhuang, Hebei, China | commercial cultivar |

the strains, and then cultured and maintained on potato dextrose agar (PDA) medium at 25 °C before testing.

**DNA extraction and polymerase chain reaction (PCR)**

Genomic DNA was extracted from fresh mycelium using the Sangon Fungus Genomic DNA Extraction Kit (Sangon Biotech, Shanghai, China). DNA concentration and purity were determined by spectrophotometry (BioSpec-nano, Shimazu, Japan) and electrophoresis in 1.4% agarose gels with known standards. PCR was performed in a final volume of 25 l, containing 30 ng of template DNA, 1.5 ml of 2.5 mM dNTP, 1.5 ml of 25 mM $MgCl_2$, 2.5 ml of 10 × buffer (0.1 mM EDTA, 100 mM KCl, 20 mM Tris-HCl, pH8.0), 1 ml of 10 mM forward and reverse primers, and 0.5 ml of 2.5 U of Taq DNA polymerase (Tiangen Biotech, Beijing, China).

Amplification was performed in a Biometra TProfessional Standard (Biometra, Gmbh, Germany). The PCR reaction was as follows: 94 °C for 10 min, 10 cycles of 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 2 min 30 s, next 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min 30 s, and a final extension step of 10 min at 72 °C, after which it was stored at 4 °C. Blank control and three negative controls were designed to improve the accuracy and reliability of the SRAP results. Three negative controls were conducted with DNA from *Coprinus comatus*, no primers, and Taq polymerase. Only the specific amplified bands compared with those of *C. comatus* were counted to ensure the specificity of the experiment. Controls with no primers and no Taq polymerase were used to verify and eliminate reagent contamination.

Amplified products were electrophoresed in 2% agarose gel with 0.5 TBE buffer at 140 V for 2.0 h and stained with ethidium bromide (0.5 mg/ml). Gels with amplification fragments were visualized and photographed in ultraviolet (UV) light using GE Image Quant Digital Imaging System (GE Healthcare Bio-Sciences AB, Sweden). 2.3. Selection of primersTo select suitable primers for studying the strains, 13 pairs of forward and reverse primers, synthesized by Sagon according to the primers reported by Li [8], were screened using 7 DNA samples. From the preliminary screening, 55 combinations of SRAP primers (8 pairs of forward and reverse primers) could amplify clear, reproducible, and polymorphic bands under optimal conditions (Table 3).

**Table 2.** SRAP primers used in the study

| FP[a] | Sequence(52 '‡32 ) | RP | sequence (5 -2 ) |
|---|---|---|---|
| Me1 | TGA GTC CAA ACC GGA TA | Em1 | GAC TGC GTA CGA ATT AAT |
| Me2 | TGA GTC CAA ACC GGA GC | Em2 | GAC TGC GTA CGA ATT TGC |
| Me3 | TGA GTC CAA ACC GGA AT | Em3 | GAC TGC GTA CGA ATT GAC |
| Me4 | TGA GTC CAA ACC GGA CC | Em4 | GAC TGC GTA CGA ATT TGA |
| Me7 | TGA GTC CAA ACC GGA CG | Em6 | GAC TGC GTA CGA ATT GCA |
| Me9 | TGA GTC CAA ACC GGA GG | Em7 | GAC TGC GTA CGA ATT CAA |
| Me10 | TGA GTC CAA ACC GGA AA | Em11 | GAC TGC GTA CGA ATT CTA |
| Me12 | TGA GTC CAA ACC GGA GA | Em13 | GAC TGC GTA CGA ATT CTG |

FR forward primer
RF reverse primer
a [8]

**Data analysis**

SRAP amplification products were compared with markers and scored in terms of binary code as present (1) or absent (0) using Image-master 1D software (Gel-Pro Analyzer, Media Cybernetics, USA). Only well-separated bands with high intensity were selected as markers. Pairwise comparisons were calculated using Jaccard's coefficient[19]. The similarity values were used to generate a consensus tree using the Unweighted Pair Group Method Arithmetic mean (UPGMA) algorithm of Nei[20]. Analyses were performed with NTSYS-pc version 2.1 [21]. Polymorphic information content (PIC), marker index (MI), qualitative nature of data, and effective marker index were calculated as follows:

**Table 3.** Primer combinations and partial genetic information generated by SRAP markers

| PC[b] | TB | PB | PP | PIC | MI | FRS (bp) |
|---|---|---|---|---|---|---|
| Me1 - Em2 | 6 | 6 | 100.0 | 0.50 | 3.00 | 100 - 1600 |
| Me1 - Em4 | 15 | 15 | 100.0 | 0.42 | 6.32 | 100 - 1800 |
| Me1 - Em6 | 11 | 10 | 90.9 | 0.45 | 4.92 | 100 - 1400 |
| Me1 - Em7 | 14 | 13 | 92.9 | 0.44 | 6.22 | 200 - 1600 |
| Me1 - Em11 | 9 | 9 | 100.0 | 0.46 | 4.12 | 100 - 2200 |
| Me1 - Em13 | 9 | 7 | 77.8 | 0.46 | 4.12 | 100 - 800 |
| Me2 - Em1 | 11 | 10 | 90.9 | 0.47 | 5.19 | 100 - 1800 |
| Me2 - Em2 | 10 | 7 | 70.0 | 0.50 | 4.99 | 100 - 1600 |
| Me2 - Em3 | 5 | 3 | 60.0 | 0.49 | 2.47 | 100 - 1200 |
| Me2 - Em4 | 15 | 11 | 73.3 | 0.46 | 6.83 | 200 - 2200 |
| Me2 - Em6 | 14 | 11 | 78.6 | 0.50 | 6.99 | 100 - 1400 |
| Me2 - Em11 | 9 | 8 | 88.9 | 0.47 | 4.19 | 100 - 1200 |
| Me2 - Em13 | 9 | 9 | 100.0 | 0.43 | 3.87 | 100 - 1600 |
| Me3 - Em1 | 8 | 7 | 87.5 | 0.44 | 3.48 | 200 - 1800 |
| Me3 - Em2 | 6 | 6 | 100.0 | 0.50 | 2.98 | 100 - 1000 |
| Me3 - Em6 | 9 | 6 | 83.3 | 0.50 | 4.49 | 200 - 1000 |
| Me3 - Em7 | 5 | 5 | 100.0 | 0.42 | 2.10 | 100 - 1200 |
| Me3 - Em11 | 12 | 12 | 100.0 | 0.44 | 5.23 | 100 - 2200 |
| Me3 - Em13 | 9 | 9 | 100.0 | 0.47 | 4.20 | 200 - 1600 |
| Me4 - Em1 | 11 | 10 | 90.9 | 0.43 | 4.70 | 100 - 2200 |
| Me4 - Em2 | 10 | 5 | 50.0 | 0.47 | 4.70 | 100 - 1600 |
| Me4 - Em3 | 8 | 5 | 62.5 | 0.42 | 3.36 | 100 - 1400 |
| Me4 - Em4 | 10 | 8 | 80.0 | 0.48 | 4.76 | 100 - 1400 |
| Me4 - Em6 | 7 | 5 | 71.4 | 0.49 | 3.44 | 100 - 1200 |
| Me4 - Em7 | 9 | 7 | 77.8 | 0.48 | 4.29 | 100 - 1600 |
| Me4 - Em11 | 7 | 6 | 85.7 | 0.44 | 3.10 | 100 - 1400 |
| Me4 - Em13 | 9 | 7 | 77.8 | 0.49 | 4.39 | 100 - 1200 |
| Me7 - Em4 | 9 | 8 | 88.9 | 0.47 | 4.23 | 100 - 2200 |
| Me7 - Em6 | 8 | 7 | 87.5 | 0.45 | 3.61 | 100 - 2000 |
| Me7 - Em7 | 6 | 6 | 100.0 | 0.37 | 2.93 | 100 - 2200 |
| Me7 - Em11 | 7 | 6 | 85.7 | 0.49 | 3.42 | 200 - 1400 |
| Me7 - Em13 | 7 | 5 | 71.4 | 0.50 | 3.47 | 200 - 2000 |
| Me9 - Em1 | 8 | 5 | 62.5 | 0.48 | 3.86 | 100 - 1000 |
| Me9 - Em2 | 10 | 8 | 80.0 | 0.48 | 4.85 | 100 - 1200 |
| Me9 - Em3 | 11 | 8 | 72.7 | 0.48 | 5.24 | 200 - 1600 |
| Me9 - Em4 | 9 | 7 | 77.8 | 0.50 | 4.49 | 100 - 1000 |
| Me9 - Em6 | 13 | 13 | 100.0 | 0.45 | 5.90 | 100 - 1200 |
| Me9 - Em7 | 9 | 6 | 66.7 | 0.50 | 4.50 | 100 - 1400 |
| Me9 - Em11 | 11 | 10 | 90.0 | 0.48 | 5.31 | 100 - 1200 |
| Me9 - Em13 | 11 | 11 | 100.0 | 0.47 | 5.20 | 100 - 1400 |
| Me10 - Em1 | 8 | 8 | 100.0 | 0.44 | 3.55 | 200 - 1400 |
| Me10 - Em2 | 14 | 13 | 92.9 | 0.50 | 7.00 | 100 - 1200 |
| Me10 - Em3 | 10 | 9 | 90.0 | 0.50 | 4.99 | 200 - 1000 |
| Me10 - Em4 | 10 | 9 | 90.0 | 0.48 | 4.76 | 200 - 1200 |
| Me10 - Em6 | 8 | 6 | 75.0 | 0.49 | 3.95 | 100 - 800 |
| Me10 - Em11 | 10 | 9 | 90.0 | 0.41 | 4.07 | 100 - 1200 |
| Me10 - EM13 | 14 | 13 | 92.9 | 0.43 | 6.02 | 100 - 1600 |
| Me12 - Em1 | 5 | 4 | 80.0 | 0.45 | 2.24 | 200 - 1000 |
| Me12 - Em2 | 8 | 7 | 87.5 | 0.45 | 3.63 | 200 - 1000 |
| Me12 - Em3 | 14 | 13 | 92.9 | 0.44 | 6.22 | 100 - 1400 |
| Me12 - Em4 | 11 | 7 | 63.6 | 0.49 | 5.39 | 100 - 1200 |

Table 3. Contd.

| | | | | | | |
|---|---|---|---|---|---|---|
| Me12 - Em6 | 17 | 15 | 88.2 | 0.50 | 8.47 | 100 - 1000 |
| Me12 - Em7 | 10 | 8 | 80.0 | 0.50 | 4.98 | 200 - 1000 |
| Me12 - Em11 | 4 | 3 | 75.0 | 0.50 | 2.00 | 200 - 1000 |
| Me12 - Em13 | 14 | 12 | 85.7 | 0.48 | 6.78 | 200 - 800 |
| Total | 533 | 453 | | | | |
| Minimum | 4 | 3 | 50 | 0.37 | 2.00 | |
| Maximum | 17 | 15 | 100 | 0.50 | 8.47 | |
| Mean | 9.69 | 8.24 | 84.67 | 0.47 | 4.54 | |

PC primer combination
TB total bands
PP percent of polymorphism
MI marker index
b [30]
PB polymorphism bands
PIC polymorphism information content
FRS fragment range size

$$PIC = 1 - \sum_{i=1}^{n} p_i^2$$

where $p_i$ is the frequency of the i th allele[22]. The PIC values measure the informativeness of a given DNA marker. In this study, these values were calculated as follows: PICi=1-Σ$(Pij)^2$, where $P_{ij}$ is the frequency of the *i*th pattern revealed by the *j*th primer summed across all patterns revealed by the primers[9].

The marker index (MI), which is the product of the total number of loci per primer (n), was calculated for each SRAP primer combination as MI = PIC x ·², where PIC is the mean PIC value, · is the number of bands, and ² is the proportion of polymorphism[23].

## RESULTS AND DISCUSSION

### Experiments of SRAP and polymorphism

After screening, 8 forward primers, 8 reverse primers, and 55 primer combinations, which could produce clear bands with good polymorphisms and reproducibility, were selected (Tables 2 and 3). The 55 primer combinations generated a total of 535 scorable bands (455 were polymorphic), with an average of 9.73 bands per primer combination. The percentage of polymorphic bands ranged between 50.0% for Me4-Em2 combination and 100% for primers of Me1-EM1, Me1-EM2, and so on, with an average of 84.67% (Table 3). The size of the detected fragments by all the primers studied in *H. marmoreus* was 100 bp to 2200 bp (Table 3 and Figure 1). Table 3 and Figure 1 show the presence of abundant polymorphism among the strains. Table 2 shows the PIC value and MI. The PIC value for SRAP ranged from 0.37 to 0.50. The mean PIC value for SRAP was 0.47. The MI for SRAP ranged from 2.00 to 8.47. The mean MI for SRAP was 4.54.

To improve the reliability, credibility, and accuracy of the study, various methods were adopted to avoid possible errors in research. The amplified fragments could be classified into weak, clear, smearing, polymorphism, robust, and specific. Only clear, polymorphism, and robust were scored; the smearing and weak bands were rejected. Primer combinations that produced less than three or several fragments were rejected because too many fragments or lesser bands would affect statistics and result accuracy. Those with poor reproducibility in repetitive experiments were not scored. Compared with the standard program (the initial 5 cycles and the next 35 cycles), the SRAP procedures were slightly modified to the initial 10 cycles and the next 30 cycles in this study. Clear amplicons and polymorphic bands were obtained with the modified procedures (Figure1).

### Phylogenetic analyses and principal coordinate analysis (PCoA) based on ISSR data

A phylogenetic tree was constructed by UPGMA cluster analysis using 535 SRAP bands from 32 tested strains (Figure 2). The collected strains of *H. marmoreus* were classified into three groups at a Jaccard's similarity coefficient of 0.49. Group A included eight storage strains: ACCC50474, ACCC51149, ACCC51532, ACCC51533, ACCC51536, ACCC51661, ACCC51583, and ACCC51800. Group B had 21 strains divided into three subgroups, namely, B1, B2, and B3 at a Jaccard's similarity coefficient of 0.54. Group C had three strains: GDGM26168,

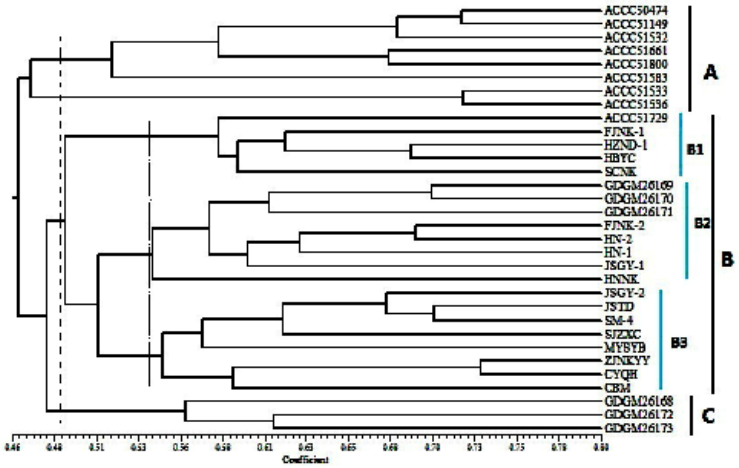


**Fig. 2.** Phylogenetic tree based on SRAP data. The *H. marmoreus* strains were classified into three subgroups at a Jaccard's similarity coefficient of 0.49. Group A included eight storage strains of ACCC. Group C had three strains: GDGM26168, GDGM26172, and GDGM26173. The remaining strains belong to Group B. The strains in Group B were classified into three subgroups at a Jaccard's similarity coefficient of 0.54

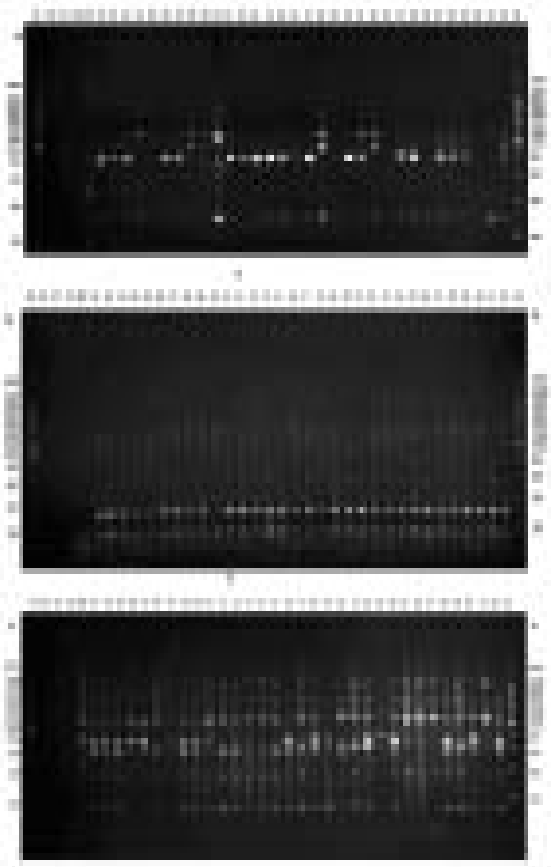

**Fig. 1.** Band profiles of *H. marmoreus* generated by SRAP primer combinations (a, ME1-EM4; b, ME4-EM7; c, ME7-EM7); The spots in NC, C1, C2, and PC correspond to negative control, no primers, no Taq polymerase, and positive control, respectively. The spots from 1 to 32 correspond to the strains of *H. marmoreus* (Table 1).

GDGM26172, and GDGM26173.

PCoA data based on the genetic similarity matrix are shown in Figure 3. The data revealed similar clusters are those with UPGMA, and could distinguish the different storage strains and commercial cultivars. The three most informative PCoA components accounted for 29.45 % of the variations observed.

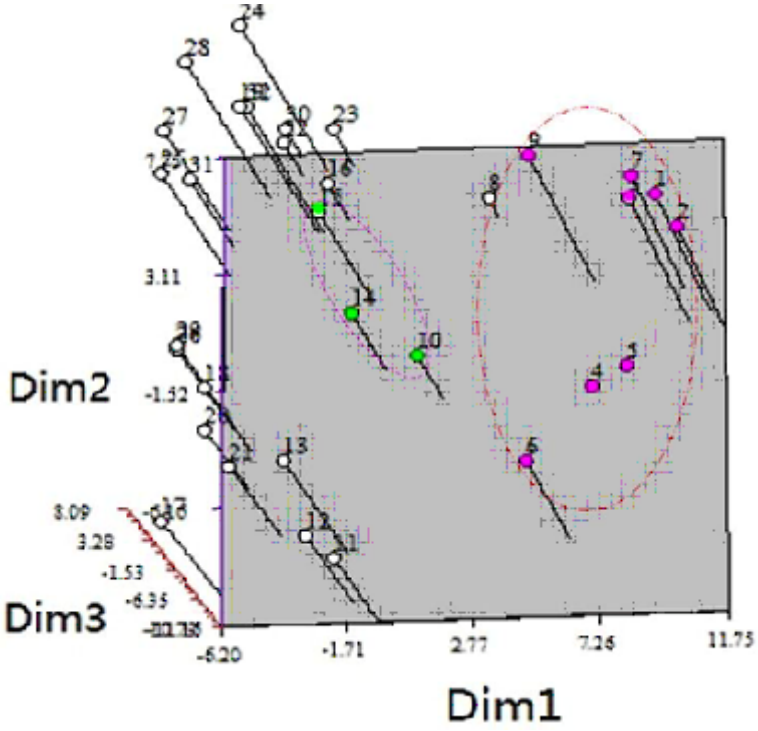


**Fig. 3.** Three-dimensional graph of the PCoA based on SRAP data. *H. marmoreus* was classified into four subgroups with groupings similar to those of UPGMA

The polymorphism level of *H. marmoreus* was medium because PIC value ranged from 0.37 to 0.5 and the mean PIC value was 0.47. PIC value that ranged from 0 to 0.25 implied that the samples had poor genetic diversity. A value of 0.25<PIC<0.5 indicated a medium level of genetic diversity among the samples. A high level of genetic diversity is present if PIC>0.50 [23-28].

Thirty-two strains were classified into three groups based on Jaccard's data and PCoA data. Group A had eight strains that were storage strains from ACCC, except ACCC51729. The other strains were attributed to Groups B and C, possibly due to the mutation and gene interchange of commercial cultivars, which were much faster and higher than those of storage strains[29]. The ACCC51729 strain might be from one of the commercial cultivars. The GDGM26168, GDGM26172, and GDGM26173 strains were clustered into one group, which indicates that they came from the same ancestor or provenance. Among the commercial cultivars, their forebear might be from two provenances. The strains of ZJNKYY, CYQH and CBM may be from the same provenance; the others might come from another.

The main aim of this study was to evaluate the genetic diversity of *H. marmoreus*, which is helpful in the selection of potential strains for breeding. SRAP is a useful method for analyzing the diversity of *H. marmoreus* in this study.

A medium level of genetic diversity and three subgroups were found among *H. marmoreus* in China. This study revealed valuable information on the relationships among different storage strains and cultivars of *H. armoreus*. SRAP markers could provide additional information on genetic study

and breeding than traditional methods. The result of our study revealed that SRAP is a useful tool for selecting potential strains and for predicting hybrid performance in the outbreeding of mushrooms.

## ACKNOWLEDGEMENT

The authors thank Dr. Li Li of Huazhong Agricultural University for his assistance in software utilization and data analysis. This work was financially supported by the Science and Technology Planning Project of Guangdong Province (STPPGD) (Nos. 2009B091300097, 2010A030100015, and 2010A090200011).

## REFERENCES

1. Lee, Y.L., Yen, M.T., and Mau, J.L. Antioxidant properties of various extracts from *Hypsizigus marmoreus. Food Chem.*, 2007; **104**(1): 1-9.
2. Jung, E.B., Jo, J.H., and Cho, S.M. Nutritional component and anticancer properties of various extracts from Haesongi mushroom (*Hypsizigus marmoreus*). *J. Korean Soc. Food Sci. Nutr.,* 2008; **37**(11): 1395-1400.
3. Akihisa, T., Franzblau, S.G., Tokuda, H., Tagata, M., Ukiya, M., Matsuzawa, T., Metori, K., Kimura, Y., Suzuki, T., and Yasukawa, K. Antitubercular activity and inhibitory effect on Epstein-Barr virus activation of sterols and polyisoprenepolyols from an edible mushroom, *Hypsizigus marmoreus. Biol. Pharm. Bull.,* 2005; **28**(6): 1117-1119.
4. Krasnopolskaya, L.M., Leontieva, M.I., Avtonomova, A.V., Isakova, E.B., Belitskii, I.V., Usov, A.I., and Bukhman, V.M. Antitumor properties of submerged cultivated biomass and extracts of medicinal mushrooms of genus *Hypsizygus* singer (*Agaricomycetideae*). *Int. J. Med. Mushrooms.*, 2008; **10**(1): 25-35.
5. Lee, Y.L., Jian, S.Y., and Mau, J.L. Composition and non-volatile taste components of *Hypsizigus marmoreus*. *LWT-Food Sci. Technol.,* 2009; **42**(2): 594-598.
6. Park, J.Y., Oh, W.J., Kwak, D.M., Kim, M.G., Seo, G.S., Hong, S.B., and Rhee, M.H. The anti-platelet activity of *Hypsizygus marmoreus* extract is involved in the suppression of intracellular calcium mobilization and integrin ±IIb²3 activation. *J. Med. Plants Res.*, 2011; **5**(11): 2369-2377.
7. Mori, K., Kobayashi, C., Tomita, T., Inatomi, S., and Ikeda, M. Antiatherosclerotic effect of the edible mushrooms *Pleurotus eryngii* (Eringi), *Grifola frondosa* (Maitake), and *Hypsizygus marmoreus* (Bunashimeji) in apolipoprotein E-deficient mice. *Nutr. Res.*, 2008; **28**(5): 335-342.
8. Li, G. and Quiros, C.F. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in *Brassica. Theor. Appl. Genet.,* 2001; **103**(2-3): 455-461.
9. Budak, H., Shearman, R.C., Parmaksiz, I., Gaussoin, R.E., Riordan, T.P., and Dweikat, I. Molecular characterization of buffalograss germplasm using sequence-related amplified polymorphism markers. *Thero. Appl. Genet.,* 2004; **108**(2): 328-334.
10. Ferriol, M., Pico, B., and Nuez, F. Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theor. Appl. Genet*., 2003; **107**(2): 271-82.
11. Cravero, V., Martín, E., and Cointry, E. Genetic diversity in *Cynara cardunculus* determined by sequence-related amplified polymorphism markers. *J. Am. Soc. Hortic. Sci*., 2007; **132**(2): 208-212.
12. Vandemark, G.J., Ariss, J.J., Bauchan, G.A., Larsen, R.C., and Hughes, T.J. Estimating genetic relationships among historical sources of alfalfa germplasm and selected cultivars with sequence related amplified polymorphisms.*Euphytica,* 2006; **152**(1): 9-16.
13. Fu, L.Z., Zhang, H.Y., Wu, X.Q., Li, H.B., Wei, H.L., Wu, Q.Q., and Wang, L.A. Evaluation of genetic diversity in *Lentinula edodes* strains using RAPD, ISSR and SRAP markers. *World J. Microb. Biot*., 2009; **26**(4): 709-716.
14. Du, P., Cui, B.C., and Dai, Y.C. Genetic diversity of wild *Auricularia polytricha* in Yunnan provinces of south-western China revealed by sequence-related amplified polymorphism (SRAP) analysis. *J. Med. Plants Res.,* 2011; **5**(8): 1374-1381.
15. Sun, S.J., Gao, W., Lin, S.Q., Zhu, J., Xie, B.G., and Lin, Z.B. Analysis of genetic diversity in *Ganoderma* population with a novel molecular marker SRAP. *Appl. Microbiol. Biot.,* 2006; **72**(3): 537-43.
16. Lee, C.Y., Park, J.E., Lee, J., Kim, J.K., and Ro, H.S. Development of new strains and related SCAR markers for an edible mushroom, *Hypsizygus marmoreus. FEMS Microbiol. Lett.,* 2012; **327**(1): 54-9.
17. Lim, Y.J., Park, J.E., Lee, C.Y., Kim, S.W., Lee, H.S., and Ro, H.S. Molecular genetic classification of *Hypsizigus marmoreus* and development of strain-specific DNA markers.

*Korean J. Mycol*., 2010; **38**(1): 34-39.
18. Wang, L., Hu, X.H., Feng, Z.Y., and Pan, Y.J. Development of AFLP markers and phylogenetic analysis in *Hypsizygus marmoreus. J. Gen. Appl. Microbiol*., 2009; **55**(1): 9-17.
19. Jaccard, P. Etude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bull Soc. Vaudoise Sci. Nat*., 1901; **37**: 547-579.
20. Nei, M. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA*, 1973; **70**(12): 3321-3323.
21. Rohlf, F.J., Numerical taxonomy and multivariate analysis system version 2.1. New York: *Applied Biostatistics Inc*., 2000; pp 1-35.
22. Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., and Rafalski, A. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. breeding*, 1996; **2**(3): 225-238.
23. Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D., and Sorrells, M.E. Optimizing parental selection for genetic linkage maps. *Genome,* 1993; **36**: 181-186.
24. Bostein, D., White, R.L., Skolnick, M., and Davis, R.W. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.,* 1980; **32**(3): 314-331.
25. Tams, S.H., Melchinger, A.E., and Bauer, E. Genetic similarity among European winter triticale elite germplasms assessed with AFLP and comparisons with SSR and pedigree data. *Plant Breeding*, 2005; **124**(2): 154-160.
26. Smith, J.S.C., Chin, E.C.L., Shu, H., Smith, O.S., Wall, S.J., Senior, M.L., Mitchell, S.E., and Ziegle, S.K.J. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays L.*) comparison with data from RFLPs and pedigree. *Theor. Appl. Genet*., 1997; **95**(1-2): 163-173.
27. Kim, H.S. and Ward, R.W. Patterns of RFLP-based genetic diversity in germplasm pools of common wheat with different geographical or breeding program origins. *Euphytica*, 2000; **115**(3): 197-208.
28. Varshney, R.K., Chabane, K., Hendre, P.S., Aggarwal, R.K., and Graner, A. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Sci*., 2007; **173**(6): 638-649.
29. Shnyreva, A.V., Belokon, I.S., Belokon, M.M., and Altukhov, I.P. Interspecific genetic variability of the oyster mushroom *Pleurotus ostreatus* as revealed by allozyme gene analysis. Genetika, 2004; **40**(8): 1068-1080.
30. Budak, H., Shearman, R.C., Gulsen, O., and Dweikat, I. Understanding ploidy complex and geographic origin of the *Buchloe dactyloides* genome using cytoplasmic and nuclear marker systems. *Theor. Appl. Genet.*, 2005; **111**(8): 1545-1552.