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SNP-Based Genetic Linkage Map and Quantitative Trait Locus Mapping Associated with the Agronomically Important Traits of *Hypsizygus marmoreus*

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ABSTRACT

White strains of *Hypsizygus marmoreus* are more difficult to cultivate than are brown strains; therefore, new white strain breeding strategies are required. Accordingly, we constructed the genetic map of *H. marmoreus* with 1996 SNP markers on 11 linkage groups (LGs) spanning 1380.49 cM. Prior to analysis, 82 backcrossed strains (HM8 lines) were generated by mating between KMCC03106-31 and the progenies of the F_1 hybrid (Hami-18 × KMCC03106-93). Using HM8, the first 23 quantitative trait loci (QTLs) of yield-related traits were detected with high limit of detection (LOD) scores (1.98–9.86). The length, thickness, and hardness of the stipe were colocated on LG 1. Especially, length of stipe and thickness of stipe were highly correlated given that the correlation coefficients were negative (-0.39, *p* value $\leq$ .01). And a typical biomodal distribution was observed for lightness of the pileus and the lightness of the pileus trait belonged to the LG 8, as did traits of earliness and mycelial growth in potato dextrose agar (PDA) medium. Therefore, results for color traits can be suggested that color is controlled by a multi-gene of one locus. The yield trait was highly negatively correlated with the traits for thickness of the stipe (-0.45, *p* value $\leq$ .01). Based on additive effects, the white strain was confirmed as recessive; however, traits of mycelial growth, lightness, and quality were inherited by backcrossed HM8 lines. This new genetic map, finely mapped QTLs, and the strong selection markers could be used in molecular breeding of *H. marmoreus*.

1. Introduction

Hypsizygus marmoreus (beech mushroom) is a species of edible and medicinal fungi native to East Asia that is now exported worldwide because of its favorable storage properties [1]. This species has different common names in different countries, e.g., neutimangadak in Korea, shimeji in Japan, and Zhengjigu in China [2]. There are two different types of cap color in *Hypsizygus marmoreus*: brown strains known as brown beech mushrooms or Buna-shimeji and white strains known as white beech mushrooms or Bunapi-shimej [3]. The scale of *H. marmoreus* development has been limited by its long cultivation period (120–150 d), high microbiological contamination levels, and limited biological efficiency [4]. Previously, a white strain was selected from a UV-irradiated brown strain by the Hokuto Corporation [5]. White strains are typically more in demand than are brown strains because the former have a less bitter taste. However, white strains are regarded as albino strains of brown strains; albino strains generally have weaker growth capacity, increased nutritional requirements, specific environmental needs, and poor resistance to adversity [6]. Therefore, new breeding strategies are needed to overcome the challenges inherent in cultivating white strains of *H. marmoreus*.

Currently, mushroom cultivars are bred using conventional selection breeding with the application of various *H. marmoreus* breeding target traits, such as mycelial incubation, high quality, and strong yield; however, most traits are characterized by polygenic control, so selected traits are limited, and selective breeding is laborious. Therefore, the identification of quantitative trait loci (QTLs) associated with these complex traits could help facilitate marker-assisted breeding. In addition, the gene linkage map is important for quantitative genetic studies and QTL candidate gene mapping, which will serve as a basis for digital breeding and gene-editing [7–9]. However, only *Agaricus bisporus* [10], *Pleurotus* spp. [8,11–14], *Lentinula* [15], *Auricularia auricula-judae* [16], and

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ⓘ Supplemental data for this article can be accessed here.

Hericium erinaceus [9] have genetic maps. *Hypsizigus marmoreus* has revealed 12 chromosomal units starting with the result that the total length of the two linkage groups (LGs) was found to be 86.2 cM by SCAR marker for the first time, but there is no research result on QTL yet [6,17].

In family-based mapping, biparental populations, such as F_2, backcrosses, double haploids, recombinant inbred lines, and near-isogenic lines, are usually used for genetic map construction and QTL mapping of crops [18]. In mushrooms, F_1 progenies, i.e., single spore isolates (SSIs) derived from the first generation, crossed with different parents are ideal for genetic mapping and are frequently used when mapping mushrooms [8,15]. In previous work, different conclusions have been drawn from genetic studies of fruit body color in edible mushrooms such as *A. bisporus* and *Flammulina velutipes* and: color traits are thought to controlled by either a single gene [19] or multiple genes [20]. The general method of backcrossing was used to construct QTL maps of agronomic traits according to fruiting body color of strain. The brown strain was used as donor and the white strain was used as the recurrent parent. In this study, we constructed a genetic map of *H. marmoreus* with single nucleotide polymorphism (SNP) discovery and genotyping through high-throughput resequencing analysis of 82 SSIs as F_1 progenies in a mapping population. In addition, we used F_1 progenies of brown and white strains for genetic map construction to increase variation.

2. Materials and methods

2.1. Fungal strains and population development

The monokaryotic *H. marmoreus* strain Hami-18 was obtained from protoclones of the dikaryotic strain Hami (KMCC03087). The monokaryotic strains compatible with KMCC03106-93 and KMCC03106-31 were isolated from KMCC03106 using mating compatibility between progenies. The F_1 hybrid strain was generated by mating between Hami-18 and KMCC03106-93. In addition, 82 progenies were collected from the F_1 hybrid strain. The 82 BC strains (HM8 lines) were generated by mating between KMCC03106-31 and the progenies of the F_1 hybrid strain (Figure S1).

2.2. Construction of the linkage map

As a reference genome, 51987-8, which is compatible with Hami-18, was sequenced [21]. The 82 progenies of the F_1 hybrid strain and three parental strains (Hami-18, KMCC03106-93, and KMCC03106-31) were resequenced using an Illumina platform at Macrogen. The DynamicTrim (phred $\geq$ 15) and LengthSort (short read length $\geq$ 25 bp) programs of SolexaQA version 1.13 (Murray Cox, New Zealand) were used to produce high-quality cleaned short reads of the resequenced sequences. The preprocessed reads were then aligned to the assembled contig (Hami-18) using the BWA (0.6.1-r104) program. SNPs were validated and detected, and consensus sequences were extracted with SAMtools (0.1.16). The SNP matrix was generated with raw SNPs and consensus sequences. After SNPs were genotyped and filtered using a minor allele frequency of $\geq$5% and <30% missing data, a genetic map was constructed using JoinMap version 4.0 (Wageningen, Netherlands) with the HAP model. The maximum likelihood mapping algorithm was applied for map construction. To assign markers to LGs, the latter were determined with a limit of detection (LOD) score of 3.0 [22–26].

2.3. Fruiting trial and phenotypic evaluation

Three parental strains (Hami, KMCC03106, and the F_1 hybrid) and HM8 lines were used in a fruiting trial for phenotypic scoring. They were cultivated in the same cultivation room at the National Institute of Horticultural and Herbal Science (Eumseong, Chungbuk, South Korea). The mycelial growth of parental strains and populations was measured after they were cultured in potato dextrose agar (PDA, Difco, USA) for 14 d. The substrates were prepared in an 850-ml polypropylene bottle with 60% *Populus* sp. sawdust, 20% wheat bran, 10% rice bran, 5% palm shell, and 5% soybean husk *via* autoclaving. The cooled substrates were inoculated using 100-ml inoculums cultured with 3–4 pieces of 1×1 cm PDA-agar covered with mycelia for 1 month. When the spawns were completed following this process, they were cultured for 90 d in a dark room maintained at 22 °C with 65% relative humidity. Mycelial growth in the spawn was measured when the mycelia had been cultured in the substrates from 10 d postinoculation. After culturing for 90 d, the outer area of spawns was removed by scraping for the formation of primordia; spawns were then placed in a light room maintained at 16 ± 1 °C with 95 ± 5% relative humidity, and light at 100 lx. When the pileus was hemispherical in shape, fruiting bodies were harvested and their traits were investigated in six replicates. The size of the pileus and stipe was measured with Mitutoyo vernier calipers; lightness was measured using a Minolta CR-400; and hardness was analyzed with a Lloyd Instrument TA Plus digital texture analyzer (Ametek, West Sussex, UK).

2.4. Statistical analyses

The 82 progenies and three parent strains of the F1 hybrid strain were used for statistical analysis by

repeating 16 individuals, respectively. The collected data for agronomic traits were subjected to a normality test and histograms of trait frequency distribution were created using "rcompanion" in the R package. The correlation coefficients for pairs of traits were obtained using Pearson's correlation analysis *via* the Performance Analytics in R. The effects of genotype on agronomic traits were determined by QTL cartographer version 2.5 (NC, USA).

2.5. QTL analysis

The QTLs for agronomic traits were scanned using composite interval mapping (CIM) model 6 (standard model) in WinQTLCart version 2.5 (NC, USA) [15,20,21]. The CIM option includes a forward and backward regression method with a walking speed of 1 cM (five control markers and a window size of 10 cM). One thousand permutation tests were conducted to set the significance threshold value ($p < 0.05$). The QTL position was considered the peak of the LOD maximum. The QTLs were denoted as follows: E, days required for earliness; H, days required for harvesting; NF, number of fruiting bodies; YE, yield; DP, diameter of pileus; TP, thickness of pileus; LS, length of stipe; TS, thickness of stipe; LiP, lightness of pileus; LiS, lightness of stipe; HP, hardness of pileus; HS, hardness of stipe; MGP, mycelial growth on the PDA; and MySp, mycelia growth in the spawn bottle. Confidence intervals (CIs) were set according to the points on the genetic map that corresponded to a decrease in the LOD score of 1 unit from the highest peak in the QTL region [27].

3. Results

3.1. Genotyping progenies and genetic map construction

The assembled contig of Hami-18 was 0.6 Gb in size with 658 scaffolds. For the clean reads of parental strains, Hami-18 was 0.79 Gb in size with 25-fold genome coverage, whereas KMCC03106-93 was 0.38 Gb in size with 10-fold genome coverage. At least 0.31 Gb of clean reads were generated for each of the 82 progenies of the F_1 hybrid strain (at least 8-fold genome coverage). The clean reads of all 82 progenies could be aligned to the assembled contig at a level >48.10%. Over 400,000 SNPs were called within these progenies. After filtering, 4722 SNPs were selected.

The genetic linkage map for *H. marmoreus* was constructed with 11 LGs and 1996 SNP markers spanning 1380.49 cM with an average interval of 0.69 cM between two adjacent markers and an average spacing of 125.50 cM. LG5 and LG11 contained the most and least markers (244 and 91), respectively. The longest and shortest LGs were LG9 and LG11 (211.12 and 62.94 cM), respectively (Table 1; Figure 1).

3.2. Trait performance

The phenotypic results for the three parental strains (Hami, KMCC03106, and the F_1 hybrid) and HM8 lines are shown in Table 2. In a quantitative comparison of KMCC03106 and Hami, the latter was harvested later and its yield was lower. Compared with KMCC03106 in terms of morphology, the pileus of Hami was small, the stipe was long and thick, and a brown cap was apparent so that the lightness of the pileus and stipe was low. Although Hami had lower hardness than KMCC03106, its mycelial growth on PDA medium occurred more rapidly. In the F_1 hybrid, the harvesting period, size of the pileus, and mycelial growth on the PDA medium were increased relative to those of Hami and KMCC03106, whereas the yield, length of the stipe, hardness of the pileus, and mycelial growth in the spawn were decreased relative to those of Hami and KMCC03106. In addition, values for the thickness of the stipe, lightness of the pileus and stipe, and hardness of the stipe were intermediate. In the HM8 lines, in which the white strain KMCC03106 (with superior yield and quality) was crossed with the F_1 hybrid, intermediate values between KMCC03106 and the F_1 hybrid were shown, except

Table 1. Characteristics of the genetic map of *Hypsizygus marmoreus*.

Linkage group	Map length (cM)	No. of SNPs	Average interval (cM)	Max interval (cM)
LG 1	143.435	176	0.815	10.491
LG 2	15.387	106	0.145	1.611
LG 3	151.696	202	0.751	16.172
LG 4	58.475	93	0.629	5.187
LG 5	94.776	244	0.388	3.945
LG 6	151.962	242	0.628	5.717
LG 7	182.663	172	1.062	15.619
LG 8	183.192	285	0.643	14.776
LG 9	211.121	252	0.838	20.554
LG 10	124.844	132	0.946	17.939
LG 11	62.942	91	0.692	4.409
Total	1,380.493	1,995	7.536	116.42
Average	125.499	181.4	0.685	10.584

Figure 1. Distribution of single nucleotide polymorphisms (SNPs) and quantitative trait loci (QTLs) responsible for the yield-related and morphology-related traits of *Hypsizygus marmoreus*. Short lines in the linkage group (LG) bars indicate SNP markers. The genetic position of SNPs is shown with a genetic ruler on the left. In total, 36 QTLs for agronomic traits are shown on the right side of the 11 LGs. The QTL regions are indicated with black rectangles. LOD-1 confidence intervals are represented by solid lines (LOD scores > 2), and QTLs with LOD scores <2.0 are represented by dashed lines.

for the length, lightness, and hardness of the stipe. Additionally, there was a substantial phenotypic difference in comparison with the parental strain, except for in the lightness of the stipe, hardness of the pileus, and hardness of the stipe. The CV value of mycelial growth in the spawn of the parental lines was not calculated because the measurements included dividing grades by the overall mycelial growth for each parental line (Table 2).

The distribution of HM8 lines showed continuous variation for all traits; however, a bimodal distribution was presented for lightness of the pileus (Figure 2) and the brown and white subgroups were divided in a 1:1 ratio. Table 3 shows the pairwise correlation coefficients by HM8 populations. The traits of earliness, days of harvesting, diameter of pileus, thickness of pileus, and thickness of stipe were positively correlated with each other. Similarly, the traits of number of fruiting bodies, yield, length of stipe, and mycelial growth in PDA medium were also positively correlated. Positive correlations were also observed for the traits of lightness of the pileus, lightness of the stipe, and mycelial growth in both the PDA medium and spawn. However, no significant correlations were found between the traits of lightness and any other traits.

3.3. QTL analysis

The empirical thresholds determined using the 1000-permutation LR test ($p<$ .05) were 1.3–2.7. Accordingly, where a QTL region exceeding the LOD threshold was not found, QTL regions for NF, YE, LiP, and HP were secured by lowering the LOD threshold to 2.0. In particular, the LOD threshold of MySP was 1.98. The QTLs for each trait in the CIM procedure are shown from one to six. The LOD scores of QTLs ranged from 1.98 to 9.86. The individual R^2 values of the QTLs ranged from 9.12 (MySp) to 52.31% (DP1). In total, 23 QTLs were identified for 14 traits. Hot spots for five QTLs that control the thickness, length, and hardness of the stipe were found at 20.00–60.70 cM on LG1. Additionally, four QTLs that control earliness, mycelial growth in PDA, and lightness of the pileus were found at 51.96–181.38 cM on LG8 (Figure 1).

3.4. QTL detection

3.4.1. Earliness and harvesting day traits

Two QTLs for earliness traits were located at positions ranging from 77.43 to 182.39 cM on LG8; these QTLs explained 38.72% and 33.37% of the phenotypic variation. The scaffold_2006_3561 was
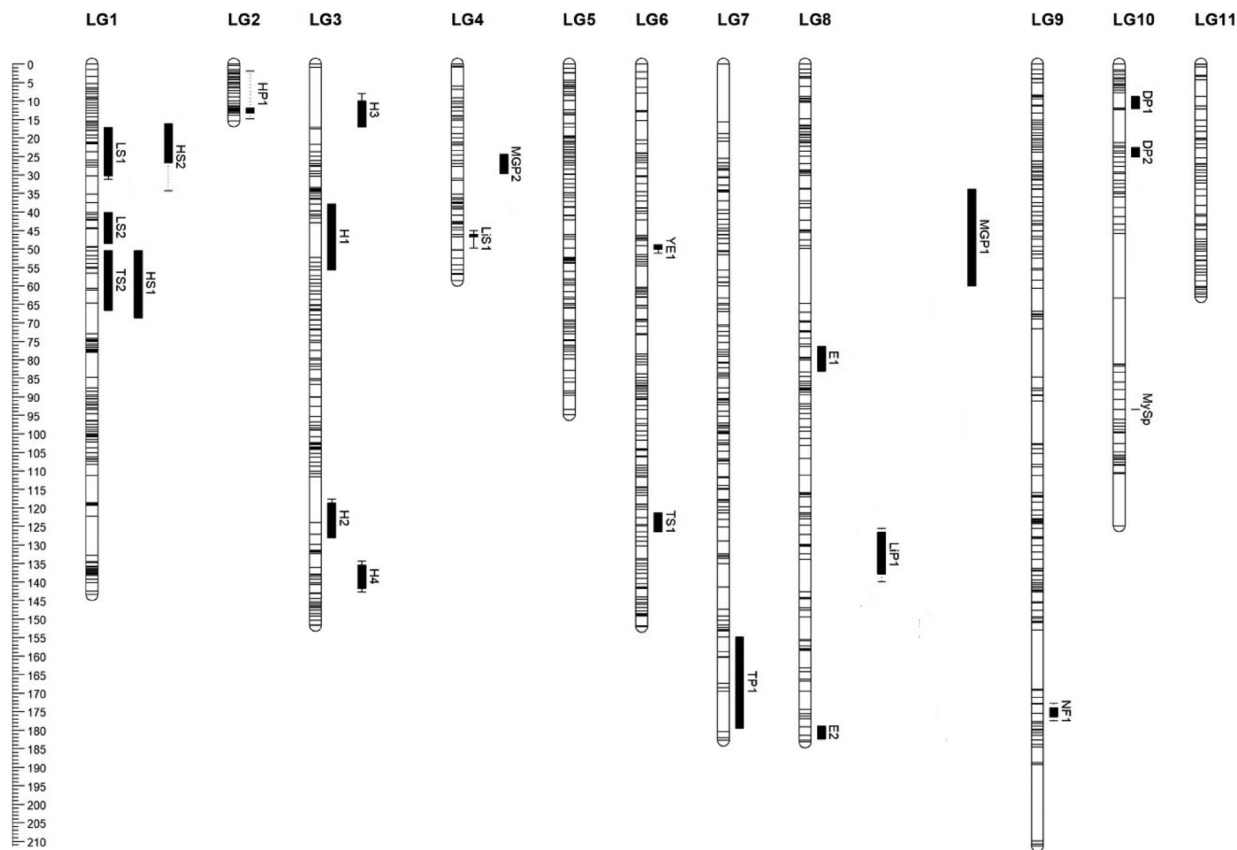
Table 2. Variation of yield-related and morphology-related traits of the HM8 lines.

Traits	Code	Parental lines {mean (SD*) [min–max]}				F1 (Hamix8088-93)	CV (%)	BC lines {mean (SD) [min–max]}	CV (%)
		Hami	CV (%)	KMCC03106	CV (%)			HM8 lines	
Earliness	E	14±0 [14.0–14.0]	0.0	11±0 [11–11]	0.0	15±0 [15–15]	0.0	13.6±3.2 [7.0–26.0]	23.5
Days of harvesting	H	28.5±1.4 [28.0–32.0]	4.9	24.7±0.5 [24.0–25.0]	1.9	31±1.4 [30.0–32.0]	4.6	27.5±4.8 [21.0–40.0]	17.3
Number of fruiting bodies	NF	31.1±0.6 [30.0–33.0]	1.9	40.6±6.2 [30.0–55.0]	15.3	21±5.7 [17.0–25.0]	26.9	32.4±13.7 [1.0–61.1]	42.3
Yield	YE	106.4±5.9 [89.6–120.8]	5.6	139±14.9 [113.6–168.0]	10.7	89.7±2.1 [88.2–91.2]	2.4	101±29.2 [4.5–156.0]	28.9
Diameter of the pileus	DP	18.5±2.1 [15.5–21.0]	11.5	18.6±1 [16.4–19.7]	5.3	21.1±2.9 [19.1–23.2]	14.0	18.9±3.9 [11.2–38.9]	20.6
Thickness of the pileus	TP	8.3±0.7 [7.4–9.2]	8.2	8.5±1.2 [6.4–10.8]	13.8	10.2±0.7 [9.7–10.7]	6.6	9.1±1.6 [6.7–14.8]	17.4
Thickness of the stipe	TS	8.3±1.4 [6.5–9.7]	17.2	6.8±0.6 [5.8–7.9]	9.4	7.5±0.2 [7.3–7.6]	2.8	7.2±1.4 [5.7–11.8]	19.6
Length of the stipe	LS	73.8±10.5 [56.3–85.5]	14.2	61.8±5.6 [50.5–70.8]	9.1	61.2±0.4 [60.9–61.5]	0.7	68.3±12.8 [28.3–95.1]	18.7
Lightness of the pileus	LiP	32.5±2.9 [29.2–36.9]	8.9	88±1.1 [85.9–89.5]	1.3	34.2±3.8 [31.6–36.9]	11.1	64.8±23.7 [32.6–91.2]	36.6
Lightness of the stipe	LiS	75.3±6.2 [66.5–83.4]	8.2	86.4±4.2 [76.3–93.2]	4.8	79.7±6.5 [75.1–84.2]	8.1	79.7±5.2 [67.4–88.5]	6.5
Hardness of the pileus	HP	4.2±0.3 [3.5–4.5]	8.0	4.7±0.7 [3.8–5.6]	15.4	3.5±0.9 [2.9–4.1]	25.1	3.8±0.9 [0.6–5.6]	23.0
Hardness of the stipe	HS	2.5±0.3 [1.93–2.99]	13.4	3.4±1 [2.0–4.9]	28.3	3.3±0.2 [3.0–3.5]	7.4	2.8±0.7 [0.4–4.6]	26.6
Mycelial growth in PDA	MGP	61.3±3.1 [57–64]	5.0	57.3±2.6 [55–61]	4.6	69.7±1.2 [68–71]	1.8	65.0±8.6 [39.0–76.7]	13.2
Mycelial growth in spawn	MGS	10	*	10.0	*	3.0	*	6.2±2.4 [0–10]	38.8

* The CV value of mycelial growth in the spawn of the parental lines was not calculated because the measurements were performed by dividing the grades by the overall mycelial growth for each parental line. Therefore Hami, KMCC03106, and F1 do not have repetition.
*SD: standard deviation

associated with earliness (LOD 7.31). Given that additive effects were more negative than positive, earliness was most likely derived from the KMCC03106.

The four QTLs for harvesting day's traits were located at positions ranging from 37.9 to 142.71 cM on LG3; these QTLs explained 20.56%, 19.66%, 18.78%, and 17.37% of the phenotypic variation, respectively. In addition, scaffold_1429_886 was associated with harvesting day (LOD 3.75). The harvesting day trait was derived from the F_1 hybrid.

3.4.2. Yield and number of fruiting bodies

One QTL for the trait yield was located at positions 48.85–51.09 cM on LG6, which was also associated with the thickness of stipe traits. This QTL explained 13.45% of the phenotypic variation and had an associated marker, namely scaffold_61_1395 (LOD 2.58). The yield traits were derived from the F_1 hybrid.

The number of fruiting bodies traits was detected on the LG6. The number of fruiting bodies trait had one QTL was located alone at positions 172.82–177.43 cM on LG9 and explained 17.84% of the phenotypic variation. The scaffold_1483_9661 was associated with the number of fruiting bodies trait (LOD 2.40); this trait was derived from KMCC03106.

3.4.3. Diameter, thickness, and hardness of the pileus

The traits for diameter, thickness, and hardness of the pileus were detected on the different LG. Two QTLs for diameter of the pileus were located at positions ranging from 9.76 to 24.01 cM on LG10 with R^2 values of 52.31% and 39.41%. And this trait was associated with mycelia growth in spawn. The associated marker was scaffold_825_309 (LOD 9.86) and the additive effect of this QTL was only positive, whereas the effects of the other QTLs were negative.

The thickness of the pileus trait was detected on one QTL located at 161.4–174.5 cM on LG7 with 32.31% of phenotypic variation explained. The scaffold_1198_25640 was associated with the thickness of the pileus.

One QTL for the hardness of the pileus trait was located at 1.92–14.79 cM on LG2 with an R^2 value of 12.29%. The marker associated with this trait was scaffold_3445_1208. Given that the additive effects of the diameter, thickness, and hardness of the pileus were more negative than positive, pileus-related traits were likely derived from KMCC03106.
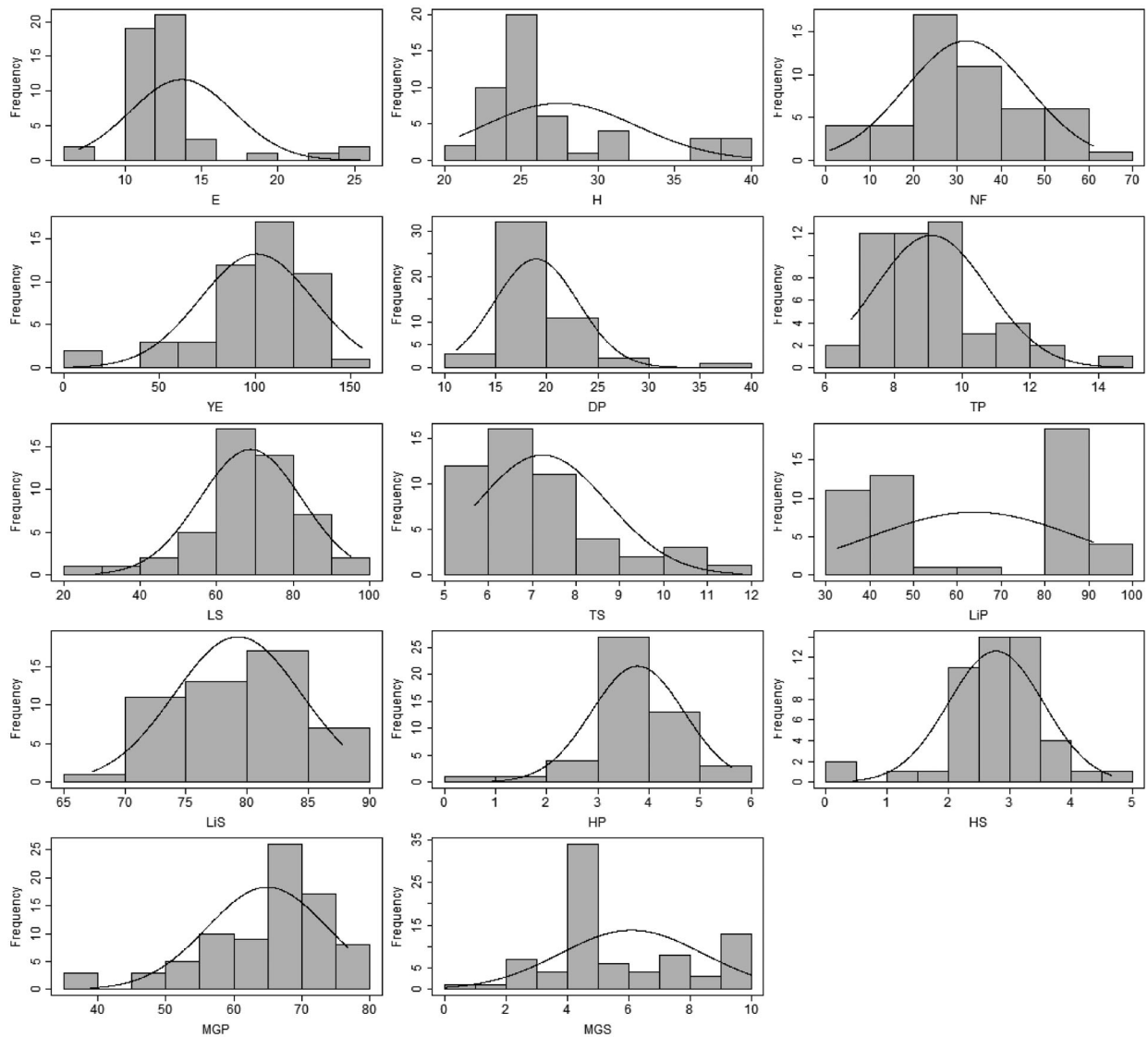
Figure 2. Frequency distribution histograms for yield-related and morphology-related traits of HM8 lines.

Table 3. Pearson's correlation coefficients for correlations between all the traits in HM8 lines.

	E	H	NF	YE	DP	TP	LS	TS	LiP	LiS	HP	HS	MGP	MGS
E	—	0.70***	−0.53**₍*₎	−0.44**	0.21	0.32*	−0.46***	0.56***	0.01	0.09	0.20	−0.28.	−0.50***	−0.16
H	0.70***	—	−0.73***	−0.62***	0.47***	0.49***	−0.56***	0.60***	−0.16	−0.0	−0.1	−0.31*	−0.47***	−0.25
NF	−0.53***	−0.73***	—	0.66***	−0.52***	−0.46***	0.62***	−0.57***	−0.10	−0.21	0.05	0.11	0.31*	0.11
YE	−0.44**	−0.62***	0.66***	—	−0.41**	−0.17	0.69***	−0.45**	−0.12	0.07	0.07	0.30*	0.47***	−0.02
DP	0.21	0.47***	−0.52***	−0.41**	—	0.40**	−0.47***	0.43**	0.18	0.05	−0.38**	−0.15	−0.15	0.05
TP	0.32*	0.49***	−0.46***	−0.17	0.40**	—	−0.14	0.58***	−0.02	0.26	−0.19	−0.10	−0.26	−0.17
LS	−0.46***	−0.56***	0.62***	0.69***	−0.47***	−0.14	—	−0.39**	−0.34*	−0.17	0.14	0.31*	0.37**	0.02
TS	0.56***	0.60***	−0.57***	−0.45**	0.43**	0.58***	−0.39**	—	0.11	0.32*	0.03	−0.32*	−0.31*	−0.06
LiP	0.01	−0.16	−0.10	−0.12	0.18	−0.02	−0.34*	0.12	—	0.66***	0.14	0.01	0.11	0.33*
LiS	0.10	−0.02	−0.21	0.07	0.05	0.26	−0.17	0.32*	0.66***	—	0.22	0.15	0.11	0.19
HP	0.20	−0.08	0.05	0.07	−0.38**	−0.19	0.14	0.03	0.14	0.22	—	0.35*	−0.00	0.02
HS	−0.28	−0.31*	0.11	0.11	−0.15	−0.10	0.31*	−0.32*	0.01	0.15	0.35*	—	0.37**	0.08
MGP	−0.50***	−0.47***	0.31*	0.47***	−0.15	−0.26	0.37**	−0.31*	0.11	0.11	−0.00	0.37**	—	0.41***
MGS	−0.16	−0.25	0.11	−0.02	0.05	−0.17	0.02	−0.06	0.33*	0.19	0.02	0.08	0.41***	—

For abbreviations, see Table 3. *$p \leq$ .05; **$p \leq$.01; ***$p \leq$.001.

3.4.4. Length, thickness, and hardness of the stipe

The traits of length, thickness, and hardness of the stipe were detected on LG1. Two QTLs for the length of the stipe were located at positions ranging from 17.24 to 48.47 cM on LG1 and they explained 23.75% and 17.39% of the variation. The scaffold_574_15414 was associated with the length of the stipe trait (LOD 3.51).

Two QTLs for the thickness of the stipe trait were found at 121.3–124.77 cM on LG6 and 50.42–66.62 cM on LG1. These QTLs explained 19.10% of the phenotypic variation on LG6 and

Table 4. Quantitative trait loci of yield-related and morphology related traits in HM8 lines.

Traits	QTL	LG	Nearest marker	Position (cM)	LOD	CI position (cM)	Additive effect	R^2 (%)	Total R^2 (%)
E	E1	LG8	scaffold_2006_3561	79.633	7.31	77.43–81.97	−6.8	38.72	72.09
	E2	LG8	scaffold_859_13570	181.38	7.14	178.91–182.39	4.9	33.37	
H	H1	LG3	scaffold_1429_886	42.876	3.75	37.9–49.89	4.3	20.56	76.37
	H2	LG3	scaffold_5901_945	123.879	3.52	117.65–128.07	4.6	19.66	
	H3	LG3	scaffold_4215_745	16.96	3.41	7.96–16.96	4.2	18.78	
	H4	LG3	scaffold_5901_5341	138.002	3.40	134.35–142.71	4.7	17.37	
NF	NF1	LG9	scaffold_1483_9661	175.423	2.40	172.82–177.43	−6.6	17.84	17.84
YE	YE1	LG6	scaffold_61_1395	49.08	2.58	48.85–51.09	12.5	13.45	13.45
DP	DP1	LG10	scaffold_825_309	11.942	9.86	9.76–11.98	10.7	52.31	91.72
	DP2	LG10	scaffold_2561_6722	23.499	7.90	22.57–24.01	−9.5	39.41	
TP	TP1	LG7	scaffold_1198_25640	160.395	4.72	161.4–174.5	−1.3	32.31	32.31
TS	TS1	LG6	scaffold_8801_507	122.59	4.20	121.3–124.77	−1.9	19.10	42.04
	TS2	LG1	scaffold_9910_726	60.7	3.76	50.42–66.62	−1.2	22.94	
LS	LS1	LG1	scaffold_574_15414	20.003	3.51	17.24–31.16	−13.3	23.75	41.14
	LS2	LG1	scaffold_9472_604	44.462	3.43	40.07–48.47	11.6	17.39	
LiP	LiP1	LG8	scaffold_7062_9277	132.41	2.58	125.63–139.94	−11.9	17.80	17.80
LiS	LiS1	LG4	scaffold_2159_2680	46.087	2.82	44.95–49.77	−4.1	17.61	17.61
HP	HP1	LG2	scaffold_3445_1208	12.552	2.34	1.92–14.79	−0.4	12.29	12.29
HS	HS1	LG1	scaffold_9910_726	60.7	3.97	50.42–66.62	0.6	24.38	39.54
	HS2	LG1	scaffold_4750_2844	26.041	2.63	16.22–34.16	−0.5	15.16	
MGP	MGP1	LG8	scaffold_49_1156	51.96	3.98	44.21–58.96	4.8	20.36	36.05
	MGP2	LG4	scaffold_416_8627	27.63	3.94	26.68–28.7	−14.9	15.69	
MGS	MySp	LG10	scaffold_3745_446	93.378	1.98	–	−0.8	9.12	9.12

22.94% of that on LG1. Scaffold_8801_507 was the marker associated with thickness of the stipe (LOD 4.20).

The hardness of the stipe trait was detected in two QTLs on LG1 at 16.22–66.62 cM. The R^2 values were 24.38% and 15.16%, respectively, and scaffold_9910_726 (LOD 3.797) was the marker associated this trait. All traits related to stipe shape and hardness were derived from KMCC03106.

3.4.5. Lightness of the pileus and stipe

One QTL for lightness of the pileus was on LG8, which was associated with earliness and mycelial growth in PDA medium. The QTLs were located at positions ranging from 125.63 to 139.94 cM on LG8 and this explained 17.80% of phenotypic variation. The associated marker for lightness of the pileus was scaffold_7062_9277 (LOD 2.58).

And one QTL for lightness of the stipe was located at positions ranging from 44.95 to 49.77 cM on LG4, which was associated with mycelial growth in PDA medium. This QTL explained 17.61% of variation and had an associated maker, scaffold_2159_2680 (LOD 2.82), for lightness of the stipe. All traits related lightness were derived from KMCC03106.

3.4.6. Mycelial growth in PDA medium and in spawn

For the trait of mycelial growth in PDA medium, one QTL was found in LG8, the other was found in LG4, which was also associated with the earliness trait with the highest LOD value (LOD 3.98). The QTLs were located at positions at 44.21–58.96 cM on LG8 and 26.68–28.7 cM on LG4. These QTLs explained 20.36% and 15.69% of the phenotypic

variation. The additive effects were more negative than positive.

One QTL for the trait of mycelial growth in spawn was located at 93.38 cM; scaffold_3745_446 (LOD 1.98) on LG10 was an associated marker and the QTL explained 9.12% of the phenotypic variation. All traits related mycelial growth were derived from KMCC03106 (Table 4).

4. Discussion

With the development of high-throughput sequencing and analysis technology, accurate genetic linkage maps have been constructed using SNPs for various mushroom species including *Pleurotus tuoliensis* [8] and *H. erinaceus* [9]. In this study, the genetic map of *H. marmoreus* was constructed with 1996 SNP markers on 11 LGs spanning 1380.49 cM (Table 1). Previously, Hu and Chu [4] produced a genetic linkage map grouped into two LGs with a total length of 86.2 cM using five sequence characterized amplified regions (SCAR) markers. In contrast, Lee et al. explored the molecular karyotype of *H. marmoreus* by contour-clamped homogeneous electric field gel electrophoresis and found that 11 chromosomal bands were separated from the dikaryotic mycelia. As the number of linkage maps and the number of chromosomal bands are equally matched, it can be inferred that the result is more accurate than the previous map [28]. But relatively small number of recombination events in biparental mapping population and high-throughput genotyping by sequencing-based approaches resulted in that 42.3% SNPs was used for construction of genetic map [29].

Analyzing the phenotyping data, the F_1 hybrid, from a cross between Hami and KMCC03106, had poor agronomic traits, e.g., low yield and a long

harvest period, other than mycelial growth. However, when crossed again with KMCC03106, which had superior traits, the resultant traits had intermediate values, with the exception of the length, lightness, and hardness of the stipe (Table 2). In mushroom breeding, repeated backcrossing can introgress the trait of interest from a donor strain into a recipient strain while limiting unfavorable linkage [30]. According to Pearson's correlation coefficients, yield increased as mycelial growth rate in the PDA medium, the number of fruiting bodies, and the length of the stipe increased. However, yield was negatively associated with earliness, days of harvesting, the diameter of the pileus, and the thickness of the stipe. The yield also increased as the values for number of fruiting bodies, length of stipe, and mycelia growth in PDA increased. In button mushrooms, the yield and number of fruiting bodies have previously been positively correlated [30], whereas the yield and length of the stipe have been positively correlated in king oyster mushrooms, while yield and earliness have been negatively correlated [14]. In particular, there were no previous results on the relationship between the lightness of the pileus and the stipe, but this study showed that the color of the pileus and the stipe were positively related to each other. Also, although it is generally said that the white strain, which is a mutant of the brown strain, has weak mycelia growth, in the study, it affects the mycelia growth positively, which is thought to be more influenced by the difference between the strains. Although not a significant difference, it was negatively related to the overall yield and harvesting day, which could support the cultivation difficulties of the white strain (Table 3). Most yield-related traits of the HM8 lines showed continuous variation, suggesting that they were under qualitative and polygenic control. However, a typical biomodal distribution was observed for lightness of the pileus, which had a 1:1 ratio consistent with the phenotype ratio of backcrossing (Figure 1). Gao et al. [31,32] reported that approximately half of the individuals were white and the other half nonwhite when the white button mushroom was crossed with the brown and white button mushroom hybrid, as shown in this study, which suggests that the color is controlled by one locus, which is unlike other traits.

As a result of this, we were able to produce the first QTL map related to agronomically important traits in *H. marmoreus*. The CV value of most traits of HM8 lines was higher than that in the parental strain, except for the lightness of the stipe and the hardness of both the pileus and stipe. It has been reported that the low CV of traits reduces the accuracy of detecting QTLs, which might be responsible for low discrimination in genotypes [11]. Therefore,

it can be said that the QTL of this result has accuracy except for some traits. The 23 QTLs were detected with high LOD scores that ranged from 1.98 to 9.86. Of the 14 traits, 7 QTLs of 4 traits were independently located on the map; the QTLs of hardness of the pileus, harvesting day, thickness of the pileus, and the number of fruiting bodies were found on LG2, LG3, LG7, and LG9, respectively. As a hot spot, five QTLs were found on LG1: two QTLs for length of stipe, two QTLs for hardness of stipe, and one QTL for thickness of stipe. Length of stipe and thickness of stipe were highly correlated given that the correlation coefficients were negative and high. This is the first study to show the relationship between stipe hardness, length, and thickness, which were highly correlated with each other. In LG 8 of the second hot spot, two QTLs for earliness, one QTL for lightness of the pileus (only found on LG8), and one QTL for mycelial growth in PDA medium were colocated on LG8. Earliness and mycelial growth in PDA medium were highly correlated given that the correlation coefficients were negative and high. Since the R^2 value of earliness was high (33.37–38.72%), the gene group in LG8 seems to be highly influential. Additionally, the trait of lightness of the stipe, which was highly correlated with lightness of the pileus, existed in LG4 along with the trait for mycelial growth. Two hypotheses exist in relation to trait color, i.e., that color traits are controlled by a single gene or by multiple genes [19,20]. The present results seem to support the latter hypothesis. Therefore, it can be suggested that color is controlled by a multi-gene of one locus. Our findings also showed that yield was colocated with thickness of the stipe on LG6; these traits were also highly correlated. Some of our results were not as expected, including for mycelial growth in spawn and the diameter of the pileus, which were colocated on LG10; specifically, the pileus-related traits belonged to different LGs, while yield and the number of fruiting bodies were also in independent positions. These results may indicate pleiotropic effects or closely linked genes as genetic factors, and they are also thought to be determined by environmental factors. In addition, in numerous edible mushrooms, including *H. marmoreus*, linkage mapping is performed using a haploid progeny (homokaryons), whereas the evaluation of numerous important agronomic traits is only possible at the dikaryotic stage after crossing with a compatible tester. This specificity can lead to inconsistencies in QTL detection (Tables 2–4). In additive effects of QTL, mycelial growth, lightness, and quality were affected by KMCC03106, a compatible tester. Additionally, the F_1 hybrid was related to quantity. Although the white strain of *H. marmoreus* was

recessive, backcrossing confirmed that strong traits were inherited from F_1.

This new genetic map and finely mapped QTLs associated with the inheritance of various traits, including mushroom production, will provide a picture of the genetic complexity of *H. marmoreus*; furthermore, identifying the location of the major loci in this species will be beneficial for further genetic analysis and for reproduction. Based on these results, molecular breeding of *H. marmoreus* will be possible using the strong selection markers identified and by applying the genetic map and fine mapping of QTL.

Disclosure statement

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Data availability statement

Data Deposition.

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