RESEARCH ARTICLE

Genetic diversity analysis of *Pleurotus ostreatus* strains based on antagonism test, ISSR markers, and agronomic traits

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Pleurotus ostreatus **is a commercially important edible fungus with high nutritional and economic value. China has the largest cultivation scale worldwide. However, non-standardized strain nomenclature during regional introduction has led to widespread homonymy and synonymy among cultivated strains, resulting in unclear genetic relationships among mainstream cultivars and insufficient scientific basis for parental selection in crossbreeding, which hinders the high-quality development of the *P. ostreatus* industry. This study comprehensively analyzed the genetic diversity and genetic relationships of 15 mainstream cultivated *P. ostreatus* strains by integrating antagonism assay, agronomic trait observation, and inter-simple sequence repeat (ISSR) molecular marker technology to clarify the genetic background and differentiation characteristics of major cultivated *P. ostreatus* strains and provide theoretical support for germplasm resource evaluation and scientific parental matching. The results showed that 87.6% of the tested strain pairs exhibited antagonistic reactions, indicating extensive genetic differences among strains. Ten screened ISSR primers amplified a total of 208 bands including 200 polymorphic bands with a polymorphic locus percentage of 96.15%. The genetic similarity coefficients among strains ranged from 0.50 to 0.86, reflecting abundant molecular genetic diversity. Significant differences were observed in agronomic traits including yield, pileus morphology, and growth cycle among the tested strains. Based on antagonism reaction patterns, the 15 tested strains were divided into 8 groups. Among them, Kejia No.1 and Xinke 101 exhibited excellent comprehensive agronomic traits including high yield, large and thick pileus. A clear antagonistic reaction was observed between the two strains, and they were assigned to different groups in ISSR cluster analysis, indicating a distant genetic distance. This study systematically clarified the genetic differentiation pattern of major cultivated *P. ostreatus* varieties and provided an important theoretical basis for the standardized evaluation of *P. ostreatus* germplasm resources and scientific parental line selection in crossbreeding.**

Keywords: *Pleurotus ostreatus*; mycelial growth; agronomic traits; antagonism; inter-simple sequence repeat; genetic diversity.

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Introduction

Pleurotus ostreatus, commonly known as oyster mushroom, is an important edible fungus worldwide. Taxonomically, it belongs to the kingdom Fungi, phylum *Basidiomycota*, order *Agaricales*, family *Pleurotaceae*, and genus *Pleurotus* [1]. It has thick flesh and a pleasant flavor and is rich in protein, amino acids, vitamins, and minerals. Thus, it has high nutritional and edible value [2, 3]. China has the largest oyster mushroom cultivation scale

globally, and this crop plays a core role in China's edible fungus production system [4].

Given its industrial importance, reliable strain identification is the foundation of oyster mushroom germplasm evaluation and breeding. To date, various methods have been established and widely used for strain identification and diversity analysis including morphological observation, physiological and biochemical markers, isozyme analysis, and molecular markers [5, 6]. Early studies mainly distinguished strains based on morphological traits, which provided a basic approach for germplasm resource assessment [7, 8]. Later, the antagonism assay became widely used for preliminary analysis of fungal genetic relationships, which was popular because of its intuitive results, simple operation, and short detection cycle [9, 10]. With the rapid progress of molecular biology, modern molecular methods have become a key direction for accurate fungal classification [11]. Inter-simple sequence repeat (ISSR) is a typical polymerase chain reaction (PCR)-based molecular marker. It has stable repeatability and high polymorphism and is commonly used in *P. ostreatus* germplasm identification. Many studies have verified its reliability in detecting genetic differences among strains [12-15]. In addition, researchers have reached a consensus that combining morphological, cytological and molecular methods can effectively improve the accuracy of strain identification [16]. However, there are still major problems restricting the high-quality development of the oyster mushroom industry and in-depth germplasm research. On the production side, non-standard strain naming during regional introduction leads to serious homonymy and synonymy among cultivated strains, which causes unclear genetic relationships among main cultivars and insufficient scientific support for parental selection in crossbreeding and finally hinders the upgrading of the oyster mushroom industry [17, 18]. On the technical side, each single identification method has obvious limitations. Morphological traits are easily affected by environmental factors such as temperature, humidity, and substrate formula, which may result in misidentification of closely related strains [9]. The antagonism assay only supports preliminary strain screening. It cannot effectively distinguish strains with similar genetic backgrounds or close genetic relationships [19]. ISSR markers also have drawbacks. As a dominant marker, it fails to fully cover functional gene loci. So, some phenotype-related genetic variations may be overlooked [20]. Currently, few studies have systematically combined the antagonism assay, ISSR markers, and agronomic trait evaluation to identify *P. ostreatus* strains and analyze their genetic diversity. This gap makes it difficult to fully and accurately reveal the genetic background and differentiation characteristics of oyster mushroom germplasm resources.

This study aimed to clarify the genetic background, genetic diversity, and differentiation characteristics of main cultivated *P. ostreatus* varieties. Fifteen mainstream cultivated *P. ostreatus* strains were selected, and an integrated analysis strategy combining antagonism assay, agronomic trait observation, and ISSR molecular markers was used to comprehensively explore the genetic relationships and diversity of the tested strains from three dimensions including cytology, phenotype, and molecular level. This study made up for the limitations of single identification methods and improved the comprehensiveness and accuracy of *P. ostreatus* germplasm evaluation. It also provided an important theoretical basis for standardized germplasm resource evaluation and scientific parental selection in crossbreeding. The findings of this study promoted the introduction, cultivation, and breeding of excellent oyster mushroom varieties and supported the high-quality development of the industry.

Materials and methods

Tested strains

Fifteen (15) *P. ostreatus* strains including 14 cultivated strains and 1 hybrid strain preserved in Edible Fungi Laboratory of Zhangzhou Institute of Technology (Zhangzhou, Fujian, China) were involved in this study, which included Xinke 101 (Shandong Academy of Agricultural Sciences, Jinan, Shandong, China), Meiping (Fujian Chengfa Agricultural Development Co., Ltd., Zhangzhou, Fujian, China), Meigaoping, Zpingzaoguan, Gaoping 88, Zaoqiu 615 (Jiangsu Jiangdu Tianda Edible Fungi Research Institute, Jiangdu, Jiangsu, China), Shuangkangheipi, Yinong 11, Kejia No.1 (Hebei Academy of Agricultural Sciences, Shijiazhuang, Hebei, China), PingguPL-9901, Huipinggu, Pingguzajiagaozhongwen (Zhangzhou Agricultural Science Research Institute, Zhangzhou, Fujian, China), Pinggu 300 (Sanming Institute of Fungi, Sanming, Fujian, China), Shuangjiang (Shanghai Academy of Agricultural Sciences, Shanghai, China), Yongrongpinggu (Quanzhou Agricultural Science Research Institute, Quanzhou, Fujian, China).

Agronomic trait evaluation

All inoculation operations were performed on a SW-CJ-1D clean bench (Suzhou Purification Equipment Co., Ltd., Suzhou, Jiangsu, China) under aseptic conditions. Potato dextrose agar (PDA) medium containing 200 g potato, 20 g glucose, 20 g agar in 1 L distilled water was used for strain activation. Mycelial plugs (5 mm in diameter) were excised from the leading edge of actively growing colonies of each strain, inoculated onto PDA plates, and incubated in the dark at 25℃ in a constant-temperature incubator until full colonization for approximately 10 days. The mother spawn and cultivation spawn medium was formulated with 53% sawdust, 30% cottonseed hull, 12% wheat bran, 2% lime, and 3% light calcium carbonate. Activated mycelia were transferred to the mother spawn medium and incubated in the dark at 22 – 24℃ for approximately 35 days until full colonization. Each mother spawn bottle was then inoculated into 25 spawn bags, which were incubated under the same dark conditions at 22 – 24℃ for approximately 35 days. The cultivation substrate consisted of 33% sawdust, 30% cottonseed hull, 20% corncob, 12% wheat bran, 2% lime, and 3% light calcium carbonate with moisture content adjusted to 62 – 64%. A completely randomized design was adopted in the cultivation experiment with 3 biological replicates for each strain and 60 cultivation bags per replicate, totaling 180 bags per strain. All bags were incubated in the dark at 22 – 24℃ until full mycelial colonization. After a 7-day post-ripening period, all cultivation bags were transferred to a commercial mushroom production greenhouse located in Zhangzhou, Fujian, China for fruiting management. Optimal conditions for fruiting body development were maintained at temperature of 18 – 22℃, relative air humidity of 85 – 95%, scattered light intensity of 200 – 1,000 lux. Water was sprayed lightly to maintain substrate surface moisture, and the greenhouse was ventilated 2 – 3 times per day for 20 – 30 min per cycle. Fruiting bodies were harvested when the pileus margin was slightly involute and not fully flattened. At the first harvest defined as the duration from primordium formation to mature fruiting body harvest, 20 fruiting bodies were randomly selected from each strain for trait measurement. Pileus color was recorded, and the long diameter, short diameter, thickness of the pileus, and stipe length were measured. The mycelial colonization period and first harvest period were documented. After two flushes of fruiting bodies were harvested, the fresh weight per bag was recorded for each strain, and digital images of fruiting body morphology were collected.

Antagonism test

The antagonism assay was performed in accordance with the National Standard NY/T 1845-2010 (Edible Fungi Strain Differentiation Identification Method) issued by the Ministry of Agriculture and Rural Affairs of the People's Republic of China (Beijing, China) [21]. After activation on PDA medium, three different strains were inoculated onto the same PDA plate in a triangular arrangement with three biological replicates for each combination. All plates were incubated in the dark at 25℃ in a constant-temperature incubator for 10 days, and the antagonistic reaction between mycelia of

Table 1. ISSR primers for identification of tested strains.

Primer No.	Primer sequence (5'-3')	Annealing temperature (℃)
807	AGA GAG AGA GAG AGA GT	52.0℃
811	GAG AGA GAG GAG AGA GAG AC	55.0℃
840	GAG AGA GAG AGA GAG AYT	56.7℃
841	GAG AGA GAG AGA GAG AYC	52.7℃
842	GAG AGA GAG AGA GAG AYG	55℃
884	HBH AGA GAG AGA GAG AG	53.0℃
885	BHB GAG AGA GAG AGA GA	57.6℃
887	DVD TCT CTC TCT CTC TC	55.5℃
888	BDB CAC ACA CAC ACA CA	53.2℃
889	DBD ACA CAC ACA CAC AC	54.7℃

Note: Degenerate bases follow the IUPAC standard coding rules. H = A/T/C. B = G/T/C. D = G/A/T. V = G/A/C. Y = C/T.

different strains was observed and recorded.

ISSR molecular marker analysis

Genomic DNA of each tested strain was extracted using a Plant Tissue Genomic DNA Extraction Kit (Sangon Biotech Co., Ltd., Shanghai, China) following manufacturer's instructions. The concentration and purity of extracted DNA were determined using a SMA4000 micro-spectrophotometer (Beijing Kaiao Technology Development Co., Ltd., Beijing, China). Qualified DNA samples were stored at -20℃ for subsequent amplification. Twenty (20) ISSR primers from the University of British Columbia (UBC) ISSR Primer Set 9 were pre-screened, and 10 primers with stable amplification bands, high polymorphism, and good repeatability were selected for formal PCR amplification (Table 1). ISSR-PCR amplification was performed in a total reaction volume of 25 µL containing 2.5 µL of 10× PCR Buffer, 2 µL of 10 µM primer, 2 µL of 10 mM dNTP, 0.2 µL of 5 U/µL Taq Plus DNA polymerase (BBI Life Sciences Corporation, Shanghai, China), 2 µL of template DNA (100 ng), and 16.3 µL of double-distilled water. Amplification reactions were carried out in a Bioer PCR-96 thermal cycler (Hangzhou Bioer Technology Co., Ltd., Hangzhou, Zhejiang, China) with 94℃ for 5 min followed by 34 cycles of 94℃ for 1 min, the corresponding primer annealing temperature for 50 s, 72℃ for 3 min and a final extension at 72℃ for 10 min. Amplification products were separated by 1.7% agarose gel electrophoresis with 1× TAE buffer. Gel images were captured using FR980 ultraviolet gel documentation system (Shanghai Furi Technology Co., Ltd., Shanghai, China).

Data analysis

One-way analysis of variance (ANOVA) was conducted using DPS 7.05 software (Zhejiang University, Hangzhou, Zhejiang, China) to test the significance of differences in agronomic traits among strains. The mean value, standard deviation, coefficient of variation, and Shannon's diversity index of each agronomic trait were calculated using Excel 2010 (Microsoft Corporation, Redmond, WA, USA). Q-type cluster analysis of quantitative traits was performed using SPSS 16.0 software (IBM, Armonk, NY, USA). For ISSR amplification results, stable and clearly visible bands were scored as 1 and absent bands as 0 to construct a binary "0 - 1" matrix. The percentage of polymorphic loci (P) was calculated to evaluate the level of genetic diversity as follows [22].

$$P = (k/n) \times 100\%$$

where P was the percentage of polymorphic loci. k was the number of polymorphic loci. n was the total number of loci. Unweighted pair-group method with arithmetic means (UPGMA) cluster analysis was performed using NTSYS-pc 2.0 (https://ntyspc.software.informer.com/2.0/) based on Sorensen's similarity coefficient to generate the genetic cluster dendrogram of the

tested strains. Principal component analysis (PCA) was performed on 7 quantitative agronomic traits after data standardization. Principal components with eigenvalues greater than 1 were extracted, and the comprehensive score of each strain was calculated by weighing the variance contribution rate of each principal component. The first principal component (PC1) mainly integrated pileus morphology and yield indicators to reflect the production performance of strains, while the second principal component (PC2) mainly integrated mycelial growth and fruiting period indicators to reflect the growth cycle characteristics of strains.

Results

Agronomic trait analysis

Significant differences in agronomic traits were observed among different *P. ostreatus* strains. For pileus color, strains with gray-black pileus included Xinke 101, Kejia No.1, and Yongrongpinggu. Meiping strain had gray pileus. Zpingzaoguan, Zaoqiu 615, and Huipinggu had light gray pileus. Shuangkangheiping, Yinong 11, and Meigaoping had gray-yellow pileus. Gaoping 88 and Pinggu 300 had gray-white pileus. No fruiting bodies were observed in strains of Pingguzajiagaozhongwen and Shuangjiang during the cultivation period. For pileus long diameter, Kejia No.1 was the largest of 84.51 mm, showing an extremely significant difference from other strains. Both Xinke 101 and Zpingzaoguan showed 82.23 mm, significantly lower than that of Kejia No.1. Yongrongpinggu was 81.63 mm, showing no significant difference from the above two strains, but differed significantly from Kejia No.1 and the remaining strains. Huipinggu and Yinong 11 had smaller pileus long diameters of 70.13 mm and 71.09 mm, respectively, with no significant difference between them, but significantly different from other strains. For pileus thickness, Xinke 101 was the thickest of 13.51 mm with no significant difference from Kejia No.1 (13.45 mm) and Meiping (12.93 mm). Huipinggu was the thinnest of 10.06 mm with no significant difference from Pinggu 300 (10.26 mm) but showed an extremely significant difference from other strains. Strains with thicker pileus generally had better storage and transportation resistance and higher commodity quality. For stipe length, Kejia No.1 was the longest of 29.11 mm followed by Xinke 101's 28.66 mm, Yongrongpinggu's 28.23 mm, and Meigaoping's 28.02 mm. There was no significant difference among these four strains, but they were significantly longer than other strains. Shuangkangheiping and Yinong 11 had the shortest stipe of 22.23 mm with no significant difference from Huipinggu (23.42 mm), but significantly shorter than other strains (Table 2).

Growth period and yield traits analysis

For mycelial colonization period, Shuangkangheiping had the shortest duration of 26.33 days, which was significantly lower than other strains. Kejia No.1 and Yinong 11 followed as 28 days and 28.33 days with no significant difference between them, but significantly different from other strains. Meigaoping had the longest mycelial colonization time of 36.67 days with a significant difference from other strains. The mycelial colonization period of Xinke 101, Zpingzaoguan, Gaoping 88, and other strains ranged from 31 to 33 days, belonging to medium maturity. For first harvest time, Kejia No.1 was the earliest of 39 days followed by Pinggu 300's 39.67 days with no significant difference between them. Huipinggu had the latest first harvest time of 50.33 days with a significant difference from other strains. For average yield per bag, Kejia No.1 was the highest (255 g) followed by Xinke 101 (251.25 g). There was no significant difference between them, but they were significantly higher than other strains. Yongrongpinggu with 237.14 g ranked third, while Shuangkangheiping had the lowest yield of 88.33 g with a significant difference from other strains. The yields of Yinong 11 (115 g), Huipinggu (128.89 g), Zpingzaoguan (130 g), and Zaoqiu 615 (142.22 g) were at a relatively low level (Table 3). The results showed that Kejia No.1 had the best comprehensive performance with high yield, early maturity, and large and thick pileus. Xinke 101 ranked second with high yield, large and

Table 2. Agronomic traits of the tested strains.

Strain	Pileus color	Pileus long diameter (mm)	Pileus short diameter (mm)	Pileus thickness (mm)	Stem length (mm)
Xinke 101	Gray black	82.23±0.69^{bB}	59.65±0.94^{aA}	13.51±0.26^{aA}	28.66±0.61^{abAB}
Meiping	Gray	76.71±1.30^{eD}	55.5±0.58^{dCD}	12.93±0.18^{abABC}	27.33±0.29^{bcdBCD}
Meigaoping	Gray yellow	80.69±1.05^{cdBC}	57.57±0.51^{bcB}	11.99±0.18^{cdDE}	28.02±0.34^{abABC}
Shuangkangheiping	Gray yellow	72.23±0.52^{hF}	49.23±0.47^{gI}	12.07±0.97^{cCDE}	22.23±0.37^{hH}
Zpingzaoguan	Light gray	82.23±0.95^{bB}	50.25±1.63^{fH}	13.23±0.46^{aAB}	24.03±0.06^{fgFG}
PingguPL-9901	Gray	74.86±1.17^{fgE}	52.38±0.66^{eFG}	11.17±0.09^{eE}	26.26±1.05^{deDE}
Yinong11	Gray yellow	71.09±0.10^{hiFG}	50.64±1.30^{fGH}	12.48±0.23^{bcBCD}	22.23±0.19^{hH}
Gaoping88	Gray white	79.55±0.61^{dC}	56.92±1.01^{cBC}	11.33±0.62^{deE}	27.87±0.20^{abcABCD}
Pingguzajiagaozhongwen	No fruiting	No fruiting	No fruiting	No fruiting	No fruiting
Zaoqiu615	Light gray	73.97±0.05^{gE}	53.04±1.08^{eEF}	11.85±0.21^{cdDE}	25.25±0.11^{efEF}
Pinggu300	Gray white	75.64±0.27^{efDE}	54.72±0.57^{dDE}	10.26±0.08^{fF}	26.49±0.12^{cedCDE}
Huipinggu	Light gray	70.13±0.12^{iG}	52.29±1.77^{eFG}	10.06±0.16^{fF}	23.42±0.27^{ghGH}
Shuangjiang	No fruiting	No fruiting	No fruiting	No fruiting	No fruiting
Kejia No.1	Gray black	84.51±0.98^{aA}	59.87±0.40^{abAB}	13.45±0.21^{aA}	29.11±0.79^{aA}
Yongrongpinggu	Gray black	81.63±0.32^{bcB}	58.64±0.85^{abAB}	13.22±0.10^{aAB}	28.23±0.35^{abAB}

Notes: Different lowercase letters in the same column indicated $P < 0.05$, while different uppercase letters in the same column indicated $P < 0.01$.

Table 3. Growth period and yield traits of the tested strains.

Strain	Mycelial colonization period (d)	First harvest time (d)	Average single bag yield (g/bag)
Xinke 101	32.33 ± 0.58^{deD}	41.67 ± 0.58^{cdefCD}	251.25 ± 22.32^{aA}
Meiping	33.00 ± 1.00^{dCD}	42.33 ± 1.15^{cdeCD}	218.13 ± 11.09^{cdBC}
Meigaoping	36.67 ± 0.58^{bB}	46.67 ± 0.58^{bAB}	232.00 ± 6.74^{bcBC}
Shuangkangheiping	26.33 ± 0.58^{iH}	41.00 ± 0^{defCD}	88.33 ± 6.41^{hG}
Zpingzaoguan	31.67 ± 1.53^{eDE}	41.67 ± 0.58^{cdefCD}	130.00 ± 5.36^{fEF}
PingguPL-9901	34.33 ± 0.58^{cC}	44.33 ± 0.58^{bcBC}	190.31 ± 7.20^{eD}
Yinong11	28.33 ± 0.58^{ghG}	42.67 ± 0.58^{cdeCD}	115.00 ± 8.24^{gF}
Gaoping88	31.67 ± 0.58^{eDE}	42.33 ± 0.58^{cdeCD}	227.78 ± 1.62^{bcdBC}
Pingguzajiagaozhongwen	No fruiting	No fruiting	No fruiting
Zaoqiu615	32.33 ± 0.58^{deD}	46.67 ± 0.58^{bAB}	142.22 ± 1.41^{fE}
Pinggu300	30.33 ± 0.58^{fEF}	39.67 ± 0.58^{efC}	214.44 ± 2.98^{dC}
Huipinggu	34.33 ± 0.58^{cC}	50.33 ± 0.58^{aA}	128.89 ± 2.09^{fEF}
Shuangjiang	29.33 ± 0.58^{fgFG}	No fruiting	No fruiting
Kejia No.1	28.00 ± 0^{hG}	39.00 ± 0^{fD}	255.00 ± 3.21^{aA}
Yongrongpinggu	30.33 ± 0.58^{fEF}	43.00 ± 5.20^{cdBCD}	237.14 ± 0.74^{bAB}

Notes: Different lowercase letters in the same column indicated $P < 0.05$, while different uppercase letters in the same column indicated $P < 0.01$.

thick pileus but slightly later maturity.

Genetic diversity analysis of agronomic traits

The genetic diversity analysis of 7 quantitative traits of tested strains showed that the coefficient of variation (CV) of the 7 quantitative traits ranged from 6.04% to 30.37% with an average CV of 11.53%, indicating rich variation in agronomic traits among the tested strains. Among them, the CV of average yield per bag was the largest (30.37%), while the CV of pileus long diameter was the smallest (6.04%). Shannon's diversity index ranged from 3.49 to 3.80 with an average value of 3.63, reflecting rich diversity, a wide genetic basis and large differences among traits. The Shannon's diversity index of mycelial colonization period was the largest (3.80), while that of stipe length was the smallest (3.49) (Table 4).

Cluster analysis of agronomic traits

Pingguzajiagaozhongwen and Shuangjiang were not included in the cluster analysis due to the absence of fruiting bodies. The cluster dendrogram based on agronomic traits showed that, when the squared Euclidean distance was set at 15, the 13 strains could be divided into 2 categories. The first category included Meiping, Pinggu 300, Meigaoping, Gaoping 88, Yongrongpinggu, Xinke 101, PingguPL-9901, and Kejia No.1. This category had relatively high average yield per bag, mostly from 214.44 –

Table 4. Genetic diversity analysis of 7 quantitative traits of tested strains.

Quantitative trait	Mean ± standard deviation	Maximum	Minimum	Coefficient of variation (CV) (%)	Shannon diversity index
pileus long diameter	77.34 ± 4.67	85.51	70.04	6.04	3.66
pileus short diameter	54.69 ± 3.66	60.32	48.74	6.69	3.66
pileus thickness	12.12 ± 1.18	13.67	9.88	9.70	3.66
Stipe length	26.09 ± 2.39	29.71	21.94	9.18	3.49
Mycelial colonization period	31.96 ± 3.53	41.00	26.00	11.04	3.80
First harvest time	43.18 ± 3.31	51.00	39.00	7.66	3.49
Average yield per bag	186.96 ± 56.77	271.39	82.39	30.37	3.62

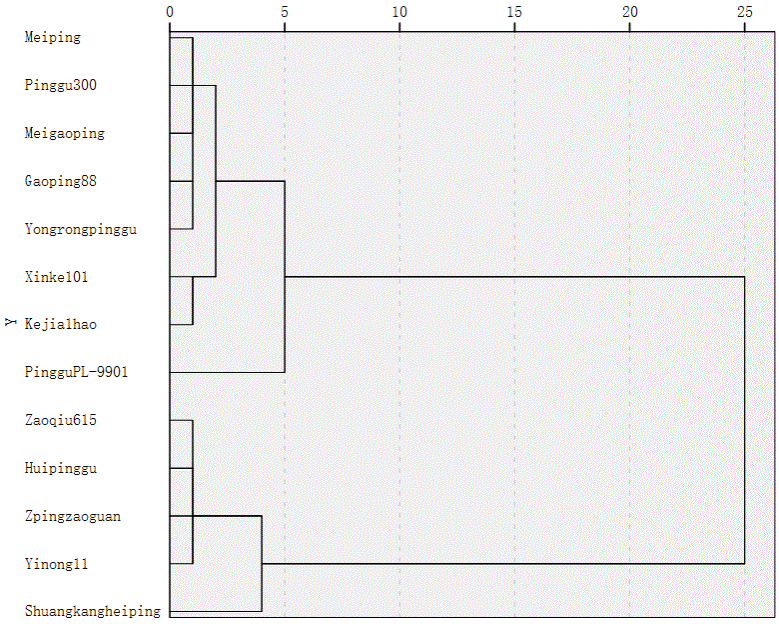

Figure 1. Cluster dendrogram based on quantitative traits of tested strains.

251.25 g/bag, belonging to the high-yield group. The pileus long and short diameters were relatively large with long diameter mostly from 75 – 82 mm and short diameter mostly from 55 – 60 mm, showing large and wide pileus. The stipe length was medium to long, mostly from 27 – 29 mm. The second category included Zaoqiu 615, Huipinggu, Zpingzaoguan, Yinong 11, and Shuangkangheipi. The average yield per bag of this category was relatively low, only 88.33 – 142.22 g/bag, belonging to the low-yield group. The pileus long and short diameters were relatively small with long diameter mostly from 70 – 74 mm and short diameter mostly from 49 – 53 mm, showing narrow and small pileus. The stipe length was relatively short, mostly from 22 – 25 mm, which was significantly shorter than that of the first category (Figure 1).

Principal component comprehensive evaluation of agronomic traits

The principal component analysis (PCA) results of 7 quantitative traits demonstrated that the eigenvalues of the first 2 principal components were both greater than 1 with a cumulative contribution rate of 84.38%. The contribution rate of the first principal component (PC1) was 56.50%, and traits with large absolute eigenvector values included pileus long diameter, pileus short diameter, pileus thickness, stipe length, and average yield per bag, which mainly reflected the production performance of strains. The contribution rate of the second principal component (PC2) was 27.88%, and traits with large absolute eigenvector values were mycelial colonization period and first harvest time, which mainly reflected the growth cycle characteristics

Table 5. Principal component analysis of quantitative traits of tested strains.

Quantitative trait	Principal component 1	Principal component 2
Pileus long diameter	0.901	-0.132
Pileus short diameter	0.927	0.208
Pileus thickness	0.552	-0.533
Stipe length	0.949	0.254
Mycelial colonization period	0.105	0.919
First harvest time	-0.433	0.799
Average yield per bag	0.937	0.244
Eigenvalue	3.955	1.952
Contribution rate (%)	56.499	27.881
Cumulative contribution rate (%)	56.499	84.380

Table 6. Component score, comprehensive score and ranking of tested strains.

Strain	Component 1 score	Component 2 score	Comprehensive score	Ranking
Xinke 101	2.58	0.14	1.50	1
Kejia No.1	3.04	-1.23	1.37	2
Meigaoping	1.26	2.33	1.36	3
Yongrongpinggu	2.00	-0.11	1.10	4
Gaoping88	1.05	0.53	0.74	5
Meiping	0.82	0.13	0.50	6
Pinggu 300	-0.07	-0.22	-0.10	7
PingguPL-9901	-0.72	1.05	-0.11	8
Zaoqiu615	-1.35	0.59	-0.60	9
Zpingzaoguan	-0.59	-1.02	-0.62	10
Huipinggu	-2.92	2.02	-1.09	11
Yinong11	-2.39	-1.73	-1.83	12
Shuangkangheiping	-2.70	-2.48	-2.21	13

of strains. Traits were sorted according to the sum of absolute values of eigenvectors in the 2 principal components. First harvest time, stipe length, and average yield per bag ranked in the top, and they also had large contribution values in each component, indicating that these 3 agronomic traits were important indicators for comprehensive phenotypic evaluation (Table 5). The comprehensive score F of each tested strain was calculated, and the results showed that Xinke 101 and Kejia No.1 had higher comprehensive scores than other strains, ranking the top 2. They also obtained high scores in PC1, belonging to high-yield strains with early maturity and long stipe (Table 6).

Analysis of antagonism reaction

The antagonism reaction matrix showed that antagonism was common among 15 *P. ostreatus* strains, and only partial strain pairs showed no antagonistic reaction. Among all 105 strain pairs, 92 pairs showed antagonistic reaction and 13 pairs showed no antagonistic reaction, indicating extensive genetic differences among the tested *P. ostreatus* strains. The antagonistic reactions were mainly manifested as obvious barrage lines between mycelia of different strains, accompanied by pigment deposition in partial combinations. Strain pairs without antagonism showed continuous and fused mycelial growth at the junction. Based on the analysis of antagonism reaction patterns among all strains, the 15 strains could be divided into 8 groups. The first group included Xinke 101, Meigaoping, Yinong 11, and Gaoping 88 with no antagonistic reaction being

Table 7. Antagonistic reactions among tested strains.

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-														
2	+	-													
3	-	+	-												
4	+	+	+	-											
5	+	+	+	+	-										
6	+	-	+	+	+	-									
7	-	+	-	+	+	+	-								
8	-	+	-	+	+	+	-	-							
9	+	+	+	+	+	+	+	+	-						
10	+	-	+	+	+	-	+	+	+	-					
11	+	+	+	+	+	+	+	+	+	+	-				
12	+	-	+	+	+	-	+	+	+	-	+	-			
13	+	+	+	+	+	+	+	+	+	+	+	+	-		
14	+	+	+	+	+	+	+	+	+	+	+	+	+	-	
15	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-

Note: + indicated obvious antagonism. - indicated no antagonism.

observed among strains in this group, indicating similar genetic background. The second group included Meiping, PingguPL-9901, Zaoqiu 615, and Huipinggu. No antagonistic reaction existed among strains in this group with similar genetic background. The third group included Kejia No.1 and Yongrongpinggu with no antagonistic reaction being found between these two strains, but both showed antagonistic reaction with all other strains. Shuangkangheipi, Zpingzaoguan, Pingguzajiagaozhongwen, Pinggu 300, and Shuangjiang showed antagonistic reaction with all other strains, indicating distant genetic relationships with other strains, thus each formed an independent group (Table 7).

ISSR polymorphism analysis

The amplified product fragment size ranged from 200 to 5,000 bp. A total of 208 bands were amplified by the 10 primers including 200 polymorphic bands with a polymorphic locus percentage of 96.15%. Each primer amplified an average of 20 polymorphic bands. The results indicated that ISSR molecular markers could amplify abundant fragments with high polymorphism for *P. ostreatus* DNA polymorphism analysis and be used to analyze the genetic relationships among different *P. ostreatus* strains.

ISSR cluster analysis

The cluster analysis dendrogram showed that the similarity coefficient of the 15 tested *P. ostreatus* strains ranged from 0.50 to 0.86. The similarity coefficient between Yongrongpinggu and Kejia No.1 was over 86%, indicating a close genetic relationship between them. When the similarity coefficient was set at 0.65, the 15 tested strains could be divided into 6 groups. The first group included Yongrongpinggu, Kejia No.1, and Shuangjiang. The second group included Pinggu 300 and Zpingzaoguan. The third group was Shuangkangheipi. The fourth group was Pingguzajiagaozhongwen. The fifth group included Huipinggu, Zaoqiu 615, PingguPL-9901, and Meiping. The sixth group included Gaoping 88, Yinong 11, Meigaoping, and Xinke 101 (Figure 2).

Discussion

Agronomic trait evaluation is a basic step for the classification and genetic diversity analysis of edible fungus germplasm resources. The variation level of these traits directly reflects the phenotypic genetic diversity of strains [23, 24]. In this study, five main agronomic traits including pileus morphology, stipe length, mycelial

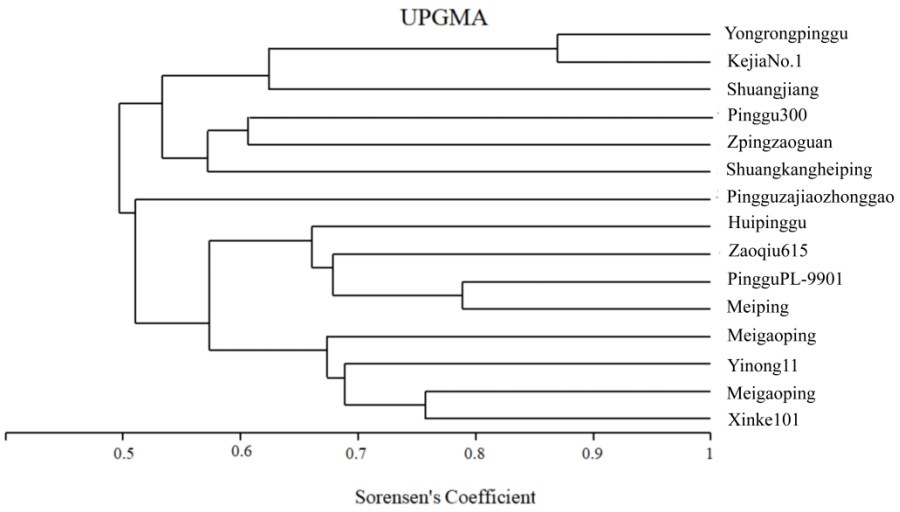

Figure 2. UPGMA dendrogram based on ISSR markers of tested *P. ostreatus* strains.

colonization period, first harvest time, and yield were measured. The average coefficient of variation of these traits was 11.53%, and the average Shannon diversity index was 3.63. These values indicated rich phenotypic genetic diversity among the tested strains, which agreed with the findings of previous studies [25, 26]. As a widely cultivated edible fungus, *P. ostreatus* has formed rich phenotypic diversity through long-term artificial domestication and natural variation [27]. Principal component analysis (PCA) was conducted to evaluate the comprehensive performance of each strain. The results showed that first harvest time, stipe length, and average yield per bag were the most important indicators for comprehensive phenotypic evaluation. Cluster analysis based on squared Euclidean distance showed that, when the distance was set at 15, the 13 fruiting strains were divided into a high-yield group and a low-yield group, where the high-yield group had large and wide pileus and medium-long stipe and the low-yield group had small and narrow pileus and short stipe. Among all strains, Kejia No.1 showed the best comprehensive performance with high yield, early maturity, and large and thick pileus. Xinke 101 ranked second and also had high yield and large thick pileus, but its maturity was slightly later. Antagonistic reaction is a type of somatic incompatibility. It is one of the main methods to evaluate genetic differences among fungal strains [28]. In this study, 92 strain pairs showed antagonistic reactions, while 13 pairs showed no antagonism. This result indicated that most tested strains had distant genetic relationships. According to the antagonistic reaction patterns, all tested strains were divided into 8 groups. Strains in the same group showed no antagonism to each other, meaning they had similar genetic backgrounds. Five strains including Shuangkangheipi, Pinggu 300, Zpingzaoguan, Pingguzajiagaozhongwen, and Shuangjiang showed antagonistic reactions to all other strains, and all had distant genetic relationships with others. So, each formed an independent group. ISSR markers can amplify DNA fragments between microsatellite sequences with specially designed primers. They can reveal genetic differences among individuals and are widely used for genetic diversity analysis of *P. ostreatus* [29]. In this study, 10 ISSR primers were used for amplification. The percentage of polymorphic loci reached 96.15%, indicating that ISSR markers could efficiently detect DNA differences among *P. ostreatus* strains. The finding was consistent with the results reported by Wu *et al*. [30]. Cluster analysis was performed based on ISSR data. The similarity coefficients of the tested strains ranged from 0.50 to 0.86, showing large genetic differences among strains. The similarity

coefficient between Yongrongpinggu and Kejia No.1 was higher than 86%, indicating that they had the closest genetic relationship. It was speculated that they might originate from the same parent or were closely related varieties, which also explained why both had excellent agronomic traits in high yield, large and wide pileus and showed no antagonistic reaction to each other. When the similarity coefficient was set at 0.65, the tested strains were divided into 6 groups by ISSR markers. The clustering results of agronomic traits (2 groups), antagonism assay (8 groups), and ISSR markers (6 groups) showed both consistency and difference [15]. For consistency, the first antagonistic group of Xinke 101, Meigaoping, Yinong 11, Gaoping 88 completely matched the 6th ISSR group. The second antagonistic group of Meiping, PingguPL-9901, Zaoqiu 615, Huipinggu completely matched the 5th ISSR group. The result verified that somatic incompatibility was highly consistent with genomic genetic differences. In addition, Yongrongpinggu and Kejia No.1 showed close relationships in all three analyses, which confirmed that genetic background played a decisive role in phenotype. For discrepancy, some strains with close genetic relationships in ISSR analysis such as Zpingzaoguan and Pinggu 300, Shuangjiang and Kejia No.1 still showed antagonistic reactions, which reflected that somatic incompatibility had stricter identification criteria. Meanwhile, Yinong 11 was clustered into the low-yield group by agronomic traits, but it belonged to the same genetic group as high-yield strains in both antagonism assay and ISSR analysis. This indicated that phenotypic traits were affected by both genotype and cultivation environment, and single phenotypic evaluation could not fully reflect the genetic background of strains [31, 32].

Overall, the combination of phenotypic traits, antagonism assay, and ISSR markers could mutually verify the genetic relationships among strains. All results jointly confirmed that the 15 tested *P. ostreatus* strains had abundant genetic diversity. Among them, Kejia No.1 and Xinke 101 showed excellent comprehensive agronomic traits such as high yield and large thick pileus. Meanwhile, a clear antagonistic reaction existed between the two strains, and they were clustered into different groups in ISSR analysis. The results indicated a distant genetic distance between them. Therefore, these two strains could be used as preferred parental materials for crossbreeding in future studies. Nevertheless, this study still had some limitations. Only 10 ISSR primers were used for molecular marker analysis, which could not fully cover the whole genome of the strains. Future research should further expand the number of tested strains, increase the screening of ISSR primers, and combine more accurate molecular marker technologies such as SSR and SNP to fully reveal the genetic differences of strains at the DNA level and provide a deeper theoretical basis for molecular marker-assisted breeding and excellent strain breeding of *P. ostreatus*.

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