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Genetic Divergence and Cluster Analysis in Shiitake Genotypes Based on Yield Related Traits with Commercial Breeding Significance to Shorten the Production Period

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ABSTRACT

Shiitake [*Lentinula edodes* (Berk) Pegler] mushroom (SM) has evolved into an important specialty food owing to its high nutritional content and medicinal properties. Progress on genetic improvement is limited due to a narrow genetic base. Genetic divergence and variability in 19 strains of SM were analyzed. Cultivation trials were conducted under environmentally controlled conditions suitable for growing *L. edodes* on synthetic logs. Mahalanobis distance (D^2) and cluster analysis using Tocher's method was used to ascertain genetic diversity. Heritability, genetic advance and components of variances were determined through variability studies. Genotypes were clustered into three major groups based on yield and yield attributing traits. The strains took an average period of 85 days from incubation to first fruiting; DMRO-388s had the shortest duration of 50 days. Days from incubation to first fruiting exhibited high heritability (97.76%) with fair genetic advance (22.83) having substantial practical utility for selection in *L. edodes*. Cluster analysis for shiitake improvement predicted the most promising crosses would be between DMRO-23×DMRO-388s, DMRO-34×DMRO-623, and DMRO-35×DMRO-388s. The DMRO-388s and DMRO-327 strains can be successfully cultivated on wheat straw based substrate due its quick colonization abilities. The pre-incubation period using a sawdust based cultivation system appears to shorten the cropping cycle of *L. edodes* which has economic implications.

KEYWORDS

Lentinula edodes; geographical diversity; incubation period; mushroom quality; quantitative traits

Shiitake mushroom [*Lenitnula edodes* (Berk.) Pegler] is a popular edible mushroom and there is increased demand for it in the global market (Royse et al., 2017). Of the several factors responsible for growth in shiitake mushroom production, standardized cultivation technologies and improved strains are important. The short duration cultivation technology of shiitake has contributed to its popularization in India (Sharma et al., 2017). The commercial potential of shiitake mushroom has not been fully

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realized. A constraint in promoting commercial production of shitake is that existing cultivated strains have a narrow genetic base. Information on the ability of introduced exotic strains to fruit under controlled conditions is scarce. Using this germplasm as a resource for genetic advancement remains unexplored.

Information on diversity in available genetic stock is a prerequisite for breeding as it forms a basis for selection of breeding material. Heterothallic, bi-factorial, mating were reported in shiitake by Takemaru (1961). Efforts to breed new cultivated strains have been made by mating strains with desirable characters. The success rate has been low due to limited available germplasm. Little work has been done on genetic diversity among shiitake strains using isozyme profiles (Fukuda and Tokimoto, 1991) and molecular studies (Liu et al., 2015; Mukhopadhyay et al., 2012; Xiang et al., 2016). Studies focusing on phenotypic and yield attributing characters, despite their significant role in management of genetic stock, are missing. It was hypothesized that performance of fungal strains in terms of stability and consistency in yield varies with production system and substrate combination. The study was undertaken to estimate genetic diversity among shiitake strains by characterizing quantitative characters of economic importance using a sawdust based substrate in a bag cultivation.

Materials and methods

Nineteen genotypes of shiitake, collected from different parts of the world, were evaluated at the Indian Council of Agricultural Research-Directorate of Mushroom Research, Solan (H.P.), India, during 2016–2017. Growth and yield related traits of each strain varied significantly (Table 1). Evaluation trials were carried on a substrate prepared by mixing sawdust of *Toona sinensis* [(A.Juss.) M.Roem] wheat bran, and gypsum in the ratio of 80:19:1 on a dry weight basis. After adjusting the moisture content of the prepared substrate to 65%, 1 kg of the substrate was filled in heat resistant polypropylene bags of 1.6 L volume. The bags were sterilized in an autoclave at 121° C and 15 psi pressure for 2 h. The spawn of the shiitake strains, prepared on wheat grains (Sharma et al., 2013), was inoculated @4% wet weight basis under aseptic conditions. Nine sterilized bags were inoculated with each strain and incubated at 25 ± 2°C till bump formation (clumps of mycelium growth that lead to fruiting). Once bump formations appeared on the surface of the substrate, the bags were peeled off and completely colonized blocks were placed in cold water (4–6°C) for 10 min. This treatment serves as a physiological shock that induces fruiting. Cold water treated blocks were transferred to a cropping room for fructification and productivity evaluation. Temperature and relative humidity were maintained at 20 ± 2°C and 85 ± 5%, respectively, till fruiting. Mature fruit bodies were harvested before the cap split.

Table 1. Cultivated strains of *L. edodes* used in the study and origin of collection.

Strain no.	Gene bank accession	Source of collection	Major traits of the strains[a]
1	DMRO-7	Philippines	S, Lm, My, G_2
2	DMRO-12	USA	S, Mm, Ly, G_3
3	DMRO-20	Nepal	S, Lm, Ly, G_3
4	DMRO-22	Nepal	S, Mm, Ly G_3
5	DMRO-23	Nepal	S, Lm, Ly, G_1
6	DMRO-34	Switzerland	S, W, Mm, My, G_2
7	DMRO-35	Switzerland	S, W, Mm, My, G_2
8	DMRO-51	Korea	S, W, Mm, Ly, G_3
9	DMRO-297	Japan	S, W, Lm, Ly, G_3
10	DMRO-327	Manipur	S, W, Mm, My, G_1
11	DMRO-328	Manipur	S, W, Mm, Ly, G_3
12	DMRO-329	Raipur	S, Mm, Lm, Ly, G_3
13	DMRO-330	Raipur	S, W, Lm, Ly, G_3
14	DMRO-331	Raipur	S, Mm, Ly, G_2
15	DMRO-356	Mycelia Belgium	S, Mm, My, G_2
16	DMRO-388	Mycelia Belgium	S, Em, Hy, G_3
17	DMRO-410	Delhi	S, W, Mm, My, G_2
18	DMRO-412	Udaipur	S, W, Mm, My, G_3
19	DMRO-623	Kerala	S, Mm, Ly, G_3

[a]S = sawdust based substrate, W = wheat straw based substrate, Em = early maturity (<60 days required for first harvest from date of spawning), Mm = medium maturity (60–90 days required for first harvest from date of spawning), Lm = late maturity (> 90 days required for first harvest from date of spawning); Ly = low yielding strains (biological efficiency (BE)<20%), My = medium yielding strains (BE 21–50%), Hy = high yielding strains (BE> 51%); G_1 = Grading based on pileus thickness, >15 mm thickness, G_2 = pileus thickness 12–15 mm, G_3 = pileus thickness <12 mm.

Data on incubation period (days from spawning to fruiting initiation), days to first harvest, pileus thickness, pileus diameter, stipe length, average fruit body weight, and total yield were recorded. Rates of radial growth rate of mycelium on saw dust extract medium and linear growth rate of mycelium on sawdust based substrate were determined.

The experiment was conducted in a randomized complete block design with three replications and three bags for each genotype in each replication. The data were analyzed with ANOVA (Panse and Sukhatme, 1964) divergence studies, group constellation, correlation coefficient, variability parameters heritability, genotypic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV). Genetic divergence was computed using Mahalanobis (1936), D^2 statistics for all possible combinations of the genotypes. Based on D^2 values, constellation of genotypes into clusters was done with Tocher's method (Rao, 1952). The relative contribution of characters toward expression of divergence was calculated following Singh and Choudhary (1977). Correlation coefficients between characters were estimated.

Results and discussion

The analysis of variance indicated differences between characters studied (Table 2). Based on Mahalanobis distance genotypes were grouped into

Table 2. Genetic parameters estimation for morphological and yield traits of *L. edodes* strains.

Observation	Linear growth rate (cm·day^{-1})	Radial growth rate (cm·day^{-1})	Incubation period (no. days)	No of days for first harvest	Pileus thickness (mm)	Pileus diameter (cm)	Stipe length (cm)	Fruit body weight (g)	Total yield (g)
GMean	0.42	0.33	76.17	84.06	11.89	7.21	4.52	19.34	85.16
PCV	25.01	26.32	14.89	12.49	26.44	21.28	23.79	33.39	71.81
GCV	23.50	25.06	14.72	12.30	24.64	17.89	19.17	25.95	68.01
h2	88.26	90.65	97.76	96.96	86.85	70.69	64.91	60.42	89.71
GA	0.19	0.16	22.83	20.97	5.63	2.23	1.44	8.04	113.01
S.E.	0.02	0.02	0.98	1.06	0.66	0.48	0.37	2.35	11.32
CD (5%)	0.06	0.04	2.78	3.00	1.87	1.36	1.05	6.67	32.18

GMean = general mean, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation, h2 = heritability, and GA = genetic advance.

three clusters using Tocher's method (Figure 1). About two-thirds of genotypes with similar agronomic traits were clustered as a part of Group I. Cluster III exhibited a wide diversity with two elite strains. A dendrogram constructed by cluster analysis separated strains in cluster I into 2 subclusters (Figure 2). Based on common divergence pattern, Group IA and Group IB were comprised of five and seven strains, respectively. The inter-cluster average D^2 value was greatest between clusters I and III, followed by average D^2 values between cluster II and III (Figure 1). Hybridization between strains from clusters I and III should result in the greatest hybrid vigor and highest number of useful segregants. The minimum inter-cluster average D^2 value was between clusters I and II indicating proximity in their genotype composition (Figure 2). Average intra-cluster distance was greatest in cluster II representing the highest diversity within the cluster.

The relative contribution of characters toward expression of divergence indicated that total yield, followed by average fruit body weight, exhibited the maximum expression to divergence (Table 3). This phenomenon justifies the importance of clustering based on economic yield. Correlation between quantitative characters indicated total yield is positively correlated with stipe length; yield was negatively correlated with incubation period and no. of days to first harvest (Table 4). Average fruit body weight was significantly, and positively, correlated with pileus diameter, pileus thickness, and stipe

Table 3. Contribution of character toward divergence.

Sl.No.	Character	Contribution toward divergence (%)
1	Linear growth rate (cm·day^{-1})	9.97
2	Radial growth rate of mycelium	10.57
3	Incubation period	6.13
4	No of days to first harvest	5.13
5	Pileus thickness	10.07
6	Pileus diameter	8.68
7	Stipe length	8.76
8	Fruit body weight	12.25
9	Total yield	28.76

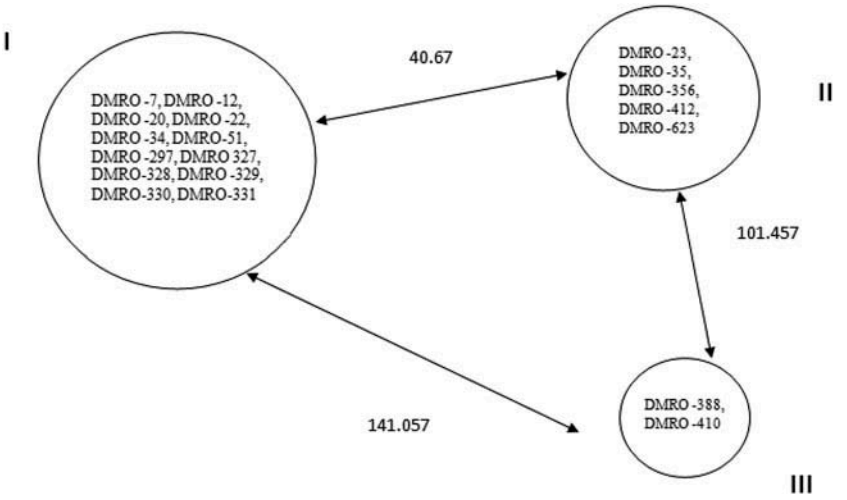

Figure 1. Divergence among clusters, I, II, III of shiitake based on D^2 values. The distance between clusters is an indication for diversity among the germplasm; the greater the distance, the better are chances for crossing strains for hybrid improved development.

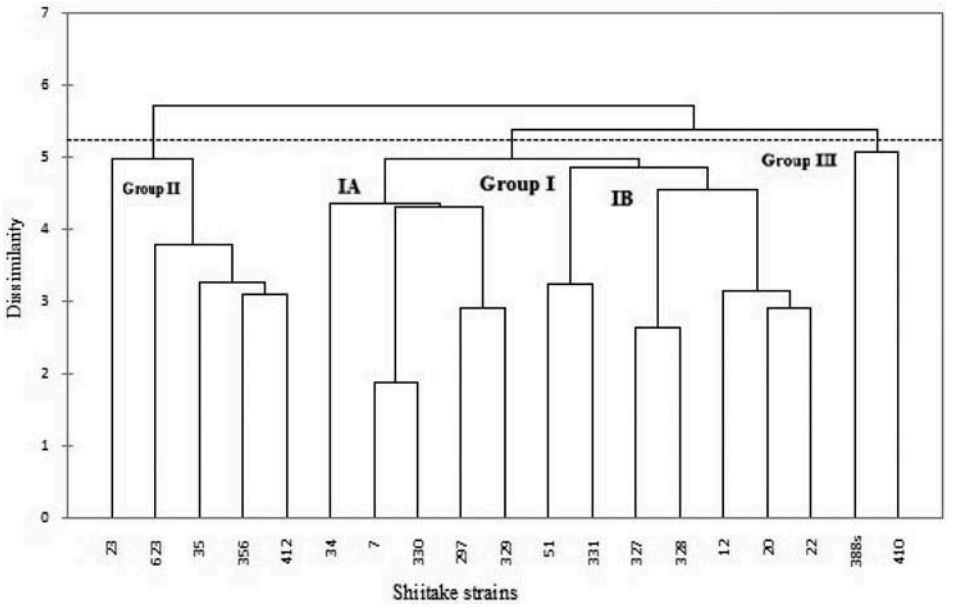

Figure 2. Clustering pattern of strains of shiitake in dendrogram based on genetic similarity coefficient.

length. The variability indicated pileus thickness had high heritability, and fair genetic advance, which can be of great utility for selection. The highest heritability was for incubation period which is important for reducing crop cycle length through selection. Average fruit body weight can be an indicator for selection because of better genetic advance value. The highest genotypic

Table 4. Correlation between characters in the study.

Character	Linear growth rate ($cm \cdot day^{-1}$) (1)	Radial growth rate of mycelium (2)	Incubation period (3)	No of days to first harvest (4)	Pileus thickness (5)	Pileus diameter (6)	Stipe length (7)	Fruit body weight (8)
2	0.373							
3	−0.397	−0.299						
4	−0.427	−0.311	0.995**					
5	−0.029	−0.045	0.466*	0.468*				
6	0.055	0.174	0.001	−0.004	0.565**			
7	0.082	0.212	−0.263	−0.289	0.287	0.720**		
8	−0.000	0.215	0.119	0.111	0.787**	0.779**	0.637**	
9 (Total yield)	0.246	0.256	−0.481*	−0.474*	−0.037	0.411	0.562**	0.324

*, ** significant at probability <0.05 and <0.01 levels, respectively.

variability was for total yield indicating the genetic stock can serve as an improved breeding population for yield improvement. Number of days to harvest and incubation period exhibited the least difference in PCV and GCV indicating more stable traits against environments. Average fruit body weight was affected more by environment than genetics.

Estimates of growth and yield are essential to identify superior genotypes. Potential parents based on high mean yield were: DMRO-34, DMRO-327, DMRO-356, DMRO-388s, DMRO-410, and DMRO-412. These potential parents can be directly used for yield improvement by selection. Based on D^2 statistics DMRO-7, DMRO-12, DMRO-20, DMRO-22, DMRO-34, DMRO-51, DMRO-297, DMRO-327, DMRO-328, DMRO-329, DMRO-330, and DMRO-331 crossed with DMRO-388s and DMRO-410 would result in maximum vigor and highest number of useful segregants for yield improvement. Although fruit quality is a key criterion for selection in shiitake, its stipe length can be an important consideration in genetic improvement. Since fruit body weight had a positive correlation with pileus diameter, and pileus thickness; stipe length can be an indirect parameter in selection for higher yield. Shiitake mushroom with shorter stipes has greater commercial demand. As the experiment was done with the wild collections, yield improvement can be the prime criteria and breeding objectives target improving higher yielding strains with shorter stipes.

The linear and radial growth rate of mycelium, incubation period and no. of days to first harvest contribute toward vegetative growth. As competitor molds act as major impediments during the initial stage of vegetative growth in synthetic log cultivation, strains with fast vegetative growth can be a significant achievement in the selection process. The strain DMRO-388s required only 50 days of pre-incubation period for fruiting and this trait has high heritability with good genetic advance. This trait can be incorporated in other superior strains to reduce the long pre-incubation period in synthetic log cultivation. The clustering pattern of strains indicated the

possibility of exploring these genotypes for hybridization and for obtaining transgressive segregants. Cluster analysis for shiitake improvement predicted the most promising crosses would be between DMRO-23×DMRO-388s, DMRO-34×DMRO-623, and DMRO-35×DMRO-388.

In shitake genetic diversity might be caused by geographical diversity in shiitake germplasm. The strong correlation between bio- and geographic-relation in shiitake strains was reported by Hibbett et al. (1995, 2001). The dendrogram constructed indicated that the shiitake has maximum diversity in east- to Southeast Asia. Genetic diversity in 88 strains of shiitake belonging to four different geographical regions indicated the population has well differentiated genetic groups separated according to geographical regions (Xiang et al., 2016). Geographically based patterns of genetic diversity could result from restricted dispersal among populations ascribed to geographic factors, which could allow time for genetic differentiations caused by mutation, drift, and selection. Sun and Lin (2003) assumed the disparity of ecological environments, and natural geography between regions, are factors impacting genetic divergence among *L. edodes* strains. Dispersal of shitake to parts of Western America and west Asia could be due to continental shift following the root of East Asia to Western America, from there to West Asia. The major diversity with respect to shiitake was in Southeast Asia. The interpretations in the present study are based on fewer accessions. More strains need to be collected from the Southeast Asia to validate the relation between geo- and genetic-diversity of shiitake. Large variation in yield attributes of shiitake within strains indicates a larger diversity. In addition to diversity not accounted for in unexplored strains, suppressed diversity due to unexpressed genes in the evaluated strains can be significant. Besides providing important information on the origin and diversity of shiitake, these findings help breeders to initiate efforts on identifying potential genetic resources for further improvement.

The shiitake strains collected from different geographical regions have mixed gene pools and expressed considerable genetic variation for yield, quality, and production period. The specific traits available in strain DMRO-23, for quality, and in DMRO-388s, for minimum pre-incubation period may be able to be brought together in to a single genotype for development of superior strains with early fruiting in shiitake.

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