JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2013. Vol. **7**(2), p. 863-869

Genetic Relationship between Growth Rate, Biomass, Germination Period and Mating Type of Monokaryons in *Lentinula edodes*

Wen-Bing Gong, Yin-Bing Bian, Rui Xu and Yang Xiao*

Key Laboratory of Agro-Microbial Resource and Development (Ministry of Agriculture), Institute of Applied Mycology, Huazhong Agricultural University, Wuhan 430 070, P.R. China.

(Received: 06 October 2012; accepted: 18 November 2012)

Monokaryons are fundamental materials for genetics and breeding research in *Lentinula edodes*. In this study, growth rate (GR), biomass (BM), germination period (GP) and mating type (MT) of 157 sporulated monokaryons from shiitake hybrid L6-26 were determined, and genetic analysis was then carried out to reveal the genetic correlation of these traits. Results showed that GR, BM and GP appeared continuous variation, which suggested these traits were controlled by polygenic system. Broad-sense heritability of GR and BM were 97.9% and 89.1% respectively, which demonstrated that GR and BM were significantly affected by genotype. The coefficients of correlation between GR, BM and GP indicated that there was significantly positive correlation between GR and BM, while GP were negatively correlated with GR and BM. Results of one-way analysis of variance (ANOVA) also showed genetic correlation between GR and MT in *L. edodes* monokaryons. Further results demonstrated that MT could explain 9.0% of total GR variation. To our knowledge, it was the first study on the genetic relationship between GR and MT in shiitake mushroom.

Key words: Shiitake mushroom, Monokaryon, Quantitative traits, Genetic correlation.

Lentinula edodes (Xianggu or shiitake mushroom), known as a valuably edible and medicinal mushroom, is the second most cultivated mushroom after *Agaricus bisporus* in term of total production[1]. It is well known the antiviral, antifungal, and antitumoral properties of *L. edodes*[2], and *L. edodes* mycelium extracts possess similar pharmacological functions[3]. For its flavor, nutrition value and medicinal properties, *L. edodes* has become part of Asian dishes culture[4]. In order to improve production, many researches have focused on genetics and breeding of *L. edodes*[5-7]. *L. edodes* is a tetrapolar heterothallic basidiomycete whose mating system is controlled by two unlinked loci (*A* and *B*) [8, 9]. Theoretically, the ratio of spores bearing four mating types (*AxBx*, *AyBy*, *AxBy* and *AyBx*) originating from the meiotic progenies should be evenly distributed as 1:1:1:1[10]. Single basidiospore forms monokaryotic mycelium after germination. Two monokaryons with different mating alleles both at *A* and *B* loci are able to fuse and generate a dikaryon which is distinguished by the presence of clamp connection.

Complex relationships among quantitative traits existed in edible mushroom. Genetic correlation analysis of quantitative traits could facilitate the understanding of underlying genetic mechanism of traits and is important for breeding research. The genetic correlation among

* To whom all correspondence should be addressed. Tel: +86-27-87282221; Fax: +86-27-87287442; E-mail: xysubmission@gmail.com

traits in edible mushroom has been widely studied, such as the relationships of vegetative growth rates on different media, production and quality of traits in *Pleurotus ostreatus*[11, 12], the relationships among yield-related traits, components of resistance in *A. bisporus*[13-15] and the correlation of quantitative traits of dikaryons in *L. edodes*[16-18].

Cross breeding via hyphal fusion between compatible monokaryons is an essential method to generate a new dikaryotic hybrid in *L. edodes*. Studies on genetic characteristics of monokaryons are helpful to choose excellent hybrid parental strains, thus improving breeding efficiency. GR, BM and GP are some of the most important traits of *L. edodes* monokaryons. GP is germination period of monokaryons which indicates initial rate of substrate colonization. GR displays growth rate of monokaryons in solid medium which has applied interest[11]. BM reflects biosynthetic ability of monokaryons in liquid medium[19].

The monokaryons are basic genetic materials in shiitake mushroom, but most studies focused on selection of excellent hybrid strains generated by mating between compatible monokaryons. There is not any report about the monokaryotic genetic correlation in *L. edodes* till now. This is the first analysis of the genetic relationship between growth rate (GR), biomass production (BM), germination period (GP) and mating types (MT) in *L. edodes* monokaryons.

MATERIALS AND METHODS

Fungal strains and determination of mating type

Mature fruiting bodies of shiitake hybrid strain L6-26 (Stored at Spawn Test Center of Huazhong Agricultural University) were picked out to get spore prints. Single spores were obtained using the dilution-plate method and incubated at 25°C[20]. Optical microscope was used to check the mycelia derived from single spore germination for absence of clamp connections to confirm their monokaryotic nature. In this study 157 monokaryons were collected. All the monokaryons were mated with four test strains and further divided into four groups by presence or the absence of clamp connections. Furthermore, the detailed mating type of each monokaryon was verified by OWE-SOJ (Oak wood extract agar-Squeezed orange juice agar) technique as previously described[20, 21].

Determination of GR, BM and GP

All the mycelia incubations were conducted in HP250S biochemical incubator (Wuhan Ruihua Instrument and Equipment Co., Ltd., China). The mycelia were recovered by inoculation on MYG petri dish (2% malt extract; 2% glucose; 2% agar; 0.1% peptone; 0.1% yeast extract) at 25°C for 15 days. Then a inoculum (8 mm diameter) generated by a hole puncher was inoculated in the center of a petri dish (90 mm diameter) containing 15 mL of MYG medium and incubated at 25°C. GP was defined as the interval days of the inoculum begin to grow on MYG medium after inoculation. After germination, the inoculum was incubated at 25°C for 15 days. GR was determined by the ratio of radial extension of mycelia colony (mm) to growth time (15 days) on MYG medium[22]. For determination of BM, a inoculum (8 mm diameter) was inoculated in a 250 mL erlenmeyer flask containing 30 mL of liquid MYG broth and incubated at 25°C for 30 days, then the mycelia were harvested and washed twice with distilled water and dried to constant weight at 80°C in DHG-9145A electrothermal blowing dry box (Shanghai Yiheng Technical Co., Ltd., China). BM was represented as the constant weight of monokaryotic mycelia. All the above-mentioned experiments were repeated three times.

Data analysis

All the data were analyzed by SPSS (Statistical Package for the Social Sciences) version 17 (SPSS Inc., Chicago, IL, USA). The ratio of the number of the monokaryons belonging to four different mating types was analyzed by chi-square test. Variation coefficients of traits were defined as the ratio of the standard deviation to the mean value. Broad-sense heritability (H^2) of the traits was assessed by one-way ANOVA using the formula $H^2 = Var(G) / Var(P)$, where $Var(G)$ is genotypic variance, and $Var(P)$ is phenotypic variance[23].

Correlation analysis was performed via the Pearson procedure[11]. In addition, MT's effect on GR, BM and GP were determined by one-way ANOVA. The contribution of MT to phenotypic variation was defined as the ratio of variation between MT groups to the total variation. Duncan's multiple range tests were further performed to analyze the difference of GR value between MT

groups. Effects of *A* mating type factor, *B* mating type factor and their interaction on GR were assessed by two-way ANOVA. The contributions of factors (*A* mating type factor, *B* mating type factor, and their interaction) to phenotypic variation were also defined as the ratio of corresponding variation between factors to total variation.

RESULTS AND DISCUSSION

Mating type determination

The four kinds of mating type were designated as *A1B1*, *A2B2, A1B2, A2B1* and their number were 37, 41, 39 and 40 respectively. The ratio of the monokaryon number belonging to the four different mating types was consistent with the theoretical 1: 1: 1: 1 ratio by chi-square test ($p = 0.974$). Also both of the monokaryon number ratios of *A1*: *A2* and *B1*: *B2* were consistent with 1: 1 ratio ($p= 0.690$ and $p= 0.811$ respectively).

Frequency distribution of GR, BM and GP

Frequency distribution of GR, BM and GP appeared as continuous variation (Fig. 1). Quantitative trait controlled by a high number of genes usually showed a continuous variation[19]. In *P. ostreatus*, the mycelium growth rate exhibited continuous variation and was presumably under the control of a polygenic genetic system[11]. In this study, the continuous variation of GR, BM and GP suggested that shiitake monokaryon growth was controlled by complex polygene.

The variation coefficients of GR, BM and GP were 46.2%, 31.3% and 45.4% respectively (Table 1), and the means of them were 1.223 mm/d, 0.202 g, and 3.688 d respectively. GR ranged from 0.267 mm/d to 3.267 mm/d, mainly distributed in 0.500-1.500 mm/d. BM ranged from 0.080 g to 0.431 g, mainly distributed in 0.100-0.300 g. GP ranged from 1 d to10 d, mainly distributed in 2-4 d. In addition, ANOVA showed that the differences of both GR and BM among strains achieved significant ($p < 0.001$). It demonstrated that the genetic background of the monokaryons had a highly significant effect on GR and BM, suggesting selection of the two traits based on genotype will be effective.

Table 1. Statistical characterization of GR, BM and GP

Trait	Range	Minimum value	Maximum value	Mean±SD	Standard deviation	Variance	Variation coefficient (%)
GR(mm/d)	3.000	0.267	3.267	1.223±0.045	0.564	0.318	46.2
BM (g)	0.351	0.080	0.431	0.202±0.005	0.063	0.004	31.3
GP(d)	9	1	10	3.688±0.134	1.675	2.806	45.4

Table 2. Correlation between GR, BM and GP

Traits	GR	BM	GP
GR	1		
BM	0.398**	1	
GP	-0.298**	-0.186*	1

Notes: ** means $p<0.01$; *means $p<0.05$

Table 3. Variance analysis of GR, BM and GP according to different MT groups

Traits	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
GR	Between Groups	4.446	3	1.482	5.013	0.002
	Within Groups	45.227	153	0.296		
	Total	49.673	156			
BM	Between Groups	0.008	3	0.003	0.639	0.591
	Within Groups	0.614	153	0.004		
	Total	0.621	156			
GP	Between Groups	16.434	3	5.478	1.990	0.118
	Within Groups	421.273	153	2.753		
	Total	437.707	156			

The broad-sense heritability (H^2) of GR and BM were 97.9% and 89.1% respectively. The high heritability may be due to the low environmental variation[22], which were consistent with other studies on *Heterobasidion annosum s. lat* and *Amylostereum areolatum*[22, 24]. We could not get the broad-sense heritability of GP because the environmental effect on GP could not be estimated for there were no differences among three parallel experiments.

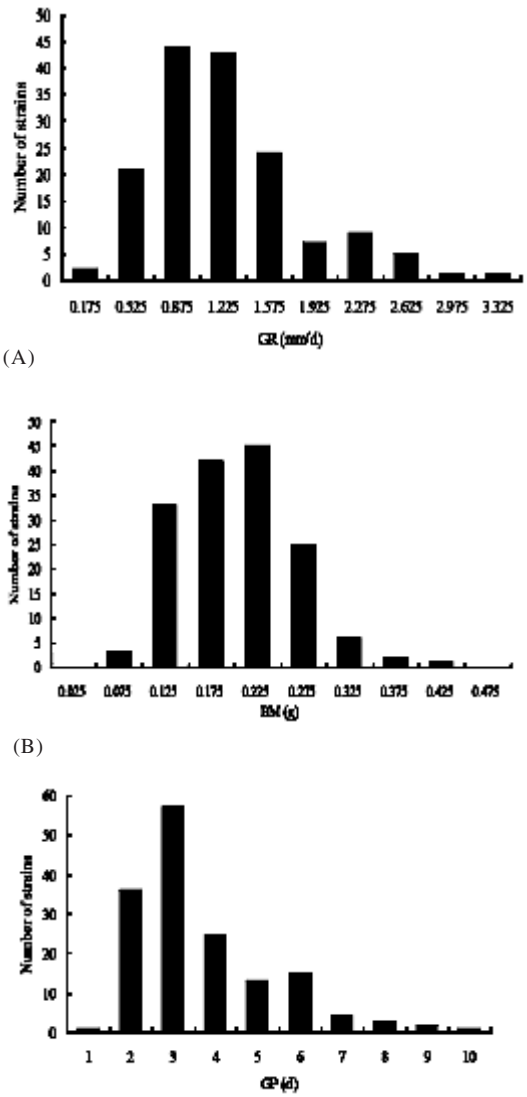
Correlation between GR, BM and GP

In the population of *L. edodes* monokaryons, GR and BM traits reflected the growth states of monokaryons in solid and liquid medium respectively. The significant positive correlation ($r = 0.398$, $p < 0.01$) between GR and BM was found (Table 2), which maybe due to the reason that rapid growing monokaryons are more efficient in utilizing substrate for biosynthesis. GP was negatively correlated with GR ($r = -0.298$, $p < 0.01$) and BM ($r = -0.186$, $p = 0.02$) (Table 2), which suggested the earlier of mycelium germination, the better of mycelium growth. It could be attributed to the fact that fast-germinating monokaryon could rapidly reproduce on the substrate and form the competition advantage. According to the correlations between GR, BM and GP, selection of fast-germinating monokaryon offered greater potential for selection of monokaryon with faster growth rate and greater synthesis ability in liquid fermentation.

Effects of MT on GR, BM and GP

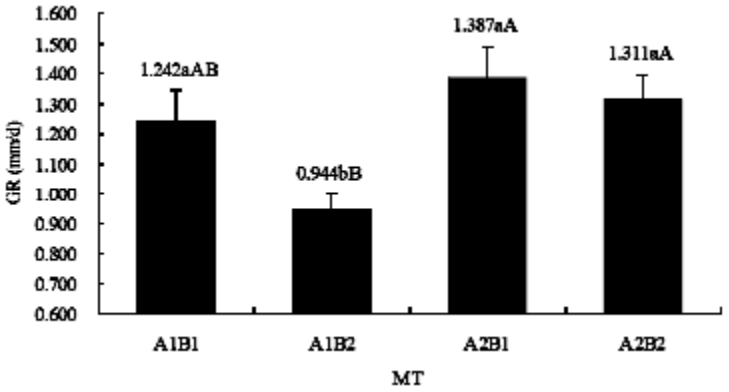
In this study, the monokaryons were divided into four groups according to their MT. The effects of MT on GR, BM and GP were analyzed using one-way ANOVA. Results indicated that BM and GP value were not significantly different between groups, while that of GR was statistically significant ($p = 0.002$) (Table 3), which means MT had a significant influence on GR. In fact it can explain 9.0% of the total GR variation.

The result of Duncan's multiple range tests was showed in Fig 2. The means of GR in *A1B1*, *A1B2*, *A2B1*, *A2B2* groups were 1.242 mm/d, 0.944 mm/d, 1.387 mm/d and 1.311 mm/d respectively. With the same *A1* mating type locus, monokaryons carrying *B1* mating type locus grew statistically significant faster than those carrying *B2* mating type locus. If the monokaryons bearing the same *A2* mating type locus, *B* locus has no significant effect on GR. If the monokaryons carrying the same *B1* mating type locus, *A* locus has no significant influence on GR. However, significant influence exists for *A* locus among the monokaryons bearing the same *B2* mating type



Notes: The class interval of the GR data is 0.35(mm/d), the value on the x-axis represents the growth rate class midpoint (A); The class interval of the BM data is 0.05(g), the value on the x-axis represents the biomass class midpoint (B); The value on the x-axis represents germination period (C).

Fig. 1. Frequency distribution of GR (A), BM (B) and GP (C)



Notes: The numbers above the column chart indicated mean of GR in different MT groups. Significant difference at 0.01 level (uppercase letters) and 0.05 level (lowercase letters) were indicated by different letter using Duncan multiple comparison. Error bars indicated standard deviations.

Fig. 2. Means of GR with four MT

locus, and monokaryons carrying *A2* mating type locus significantly grew faster than those carrying *A1* mating type locus (Fig. 2).

In addition, the means of GR for monokaryons that contained the *A1*, *A2*, *B1* and *B2* alleles were 1.089mm/d, 1.348 mm/d, 1.317 mm/d and 1.132 mm/d respectively. The results of two-way ANOVA indicated *A* mating type locus had significant effect on GR, it contributed 5.2% of the total GR variation. Monokaryons bearing the *A2* (*A2B1+A2B2*) mating type locus grew significantly faster than those bearing the *A1* (*A1B1+A1B2*) mating type locus ($p = 0.004$). Furthermore, monokaryons bearing the *B1* (*A1B1+A2B1*) mating type locus grew significantly faster than those bearing the *B2* (*A1B2+A2B2*) mating type locus ($p = 0.033$). *B* mating type locus explained 2.8% of the total GR variation. No significant interaction was found between *A* and *B* mating type loci ($p = 0.204$), and the contribution of *A*×*B* interaction was 1%.

Mating type factors are important genetic markers and play a conclusive role in determining the exchange of nuclei between compatible haploid mycelia to form dikaryotic mycelium. In *P. ostreatus*, the monokaryons bearing the *A2* mating allele grew faster than those bearing *matA1* allele, and significant differences were also surveyed between matBα1 and matBα2 alleles in monokaryons carrying the *A2* allele[25]. The phenomenon of mycelial growth affected both by mating type factor *A* and *B* was also found in *Schizophyllum commune*, in fact, growth rate was linked to *A* mating type factor, and loosely linked to *B* mating type factor in this fungi[26]. In addition, the similar relationship between mycelial growth and sexual recognition had also been studied in *A. bisporus*, *H. annosum s. lat* and *A. areolatum*[22, 24, 27].

Larraya *et al*. speculated that genetic determinants affecting growth rate and mating type genes were kept together in Agaricales[25]. Our study also showed similar results in *L. edodes*. However, the underlying mechanism for this association is still unknown. The QTL (quantitative trait loci) controlling growth rate was not mapped in the region of mating type genes *A* and *B* in *P. ostreatus* [25]. Conversely, the putative QTL associated with mycelial growth were positioned near the recognition loci (*mat-A* and *het-A*) in *A. areolatum*[24]. As a quantitative trait, the mycelial growth rate is controlled by a polygenic genetic system and co-influenced both by genetic background and environmental factors. It is insufficient to select genotype only using phenotype data. QTL mapping is an alternative method for study on the quantitative trait and was successfully used to map QTL in mushroom, such as QTL controlling the growth rate in the mushroom *A. bisporus*[28], *P. ostreatus*[11] and in the dikaryon on PDA medium in *L. edodes*[6]. The present study provided basic phenotype information for *L. edodes* QTL mapping that is now in progress in our lab. Based on a genetic

map of *L. edodes*, a putative QTL controlling growth rate of monokaryons in MYG medium was mapped near the *A* mating type locus (our unpublished data).

CONCLUSIONS

The correlations between GR, BM, GP and MT of *L. edodes* monokaryons were evaluated in the present study. Results showed that GR had a significant positive correlation with BM, but GP was negatively correlated with GR and BM. MT had a significant influence on GR. This is the first evidence on the correlation between mycelial growth rate and the mating type in *L. edodes* monokaryons. For complex phenotype characteristics, it is difficult to directly select suitable monokaryons from massive ones for breeding study. The survey of genetic relationship between important traits might facilitate the understanding of genetic architecture of monokaryotic mycelium growth and form basis for selection of appropriate monokaryons for genetic and breeding research in *L. edodes*.

ACKNOWLEDGEMENTS

This work was financially supported by National Natural Science Foundation of China (No. 31000929)

REFERENCES

1. Xiao, Y., Liu, W., Dai, Y.H., Fu, C., Bian, Y.B. Using SSR markers to evaluate the genetic diversity of *Lentinula edodes*' natural germplasm in China. *World. J. Microbiol. Biotechnol*., 2010; **26**(3): 527 – 536.
2. Bisen, P.S., Baghel, R.K., Sanodiya, B.S., Thakur, G.S., Prasad, G.B. *Lentinus edodes*: a macrofungus with pharmacological activities. *Curr. Med. Chem*., 2010; **17**(22): 2419 – 2430.
3. Shen, J., Tanida, M., Fujisaki, Y., Horii, Y., Hashimoto, K., Nagai, K. Effect of the culture extract of *Lentinus edodes* mycelia on splenic sympathetic activity and cancer cell proliferation. *Auton. Neurosci*., 2009; **145**(1-2): 50 – 54.
4. Turlo, J., Gutkowska, B., Herold, F., Krzyczkowski, W., Blazewicz, A., Kocjan, R. Optimizing vitamin B_{12} biosynthesis by mycelial cultures of *Lentinula edodes* (Berk.) Pegl. *Enzyme. Microb. Technol*., 2008; **43**(4-5): 369 – 374.
5. Kwan, H.S., Xu, H.L. Construction of a genetic linkage map of shiitake mushroom *Lentinula edodes* strain L-54. *J. Biochem. Mol. Biol*., 2002; **35**(5): 465 – 471.
6. Miyazaki, K., Huang, F.X., Zhang, B.X., Shiraishi, S., Sakai, M., Shimaya, C., Shishido, K. Genetic map of a basidiomycete fungus, *Lentinula edodes* (shiitake mushroom), constructed by tetrad analysis. *Breed. Sci*., 2008; **58**(1): 23 – 30.
7. Miyazaki, K., Sakai, M., Miyazaki, Y. Mapping of genes abundantly expressed during fruiting body formation of *Lentinula edodes*. *Breed. Sci*., 2010; **60**(1): 81 – 86.
8. Murakami, S., Takemaru, T. "Puff" mutation induced by UV irradiation in *Lentinus edodes* (Berk.) Sing. *Rep. Tottori. Mycol. Inst*., 1975; **12**: 47 – 51.
9. Tokimoto, K., Komatsu, M., Takemaru, T. Incompatibility factors in the natural population of *Lentinula edodes* in Japan. *Rep. Tottori. Mycol. Inst*., 1973; **10**: 371 – 376.
10. Cheng, S.M., Lin, F.X. Genetic analysis of distorted segregation ratio of mating types among basidiospores in *Lentinula edodes*. *Scientia. Agricultura. Sinica*., 2008; **7**(4): 415 – 422.
11. Larraya, L.M., Idareta, E., Arana, D., Ritter, E., Pisabarro, A.G., Ramírez, L. Quantitative trait loci controlling vegetative growth rate in the edible basidiomycete *Pleurotus ostreatus*. *Appl. Environ. Microbiol*., 2002; **68**(3): 1109 – 1114.
12. Larraya, L.M., Alfonso, M., Pisabarro, A.G., Ramírez, L. Mapping of genomic regions (quantitative trait loci) controlling production and quality in industrial cultures of the edible basidiomycete *Pleurotus ostreatus*. *Appl. Environ. Microbiol*., 2003; **69**(6): 3617 – 3625.
13. Foulongne-Oriol, M., Rodier, A., Rousseau, T., Largeteau, M., Savoie, J.M. Quantitative genetics to dissect the fungal-fungal interaction between *Lecanicillium fungicola* and the white button mushroom *Agaricus bisporus*. *Fungal. Biol*., 2011; **115**(4-5): 421 – 431.
14. Foulongne-Oriol, M., Rodier, A., Rousseau, T., Savoie, J.M. Quantitative trait locus mapping of yield-related components and oligogenic control of the cap color of the button mushroom, *Agaricus bisporus*. *Appl. Environ. Microbiol*., 2012a; **78**(7): 2422 – 2434.
15. Foulongne-Oriol, M., Rodier, A., Savoie, J.M. Relationship between yield components and partial resistance to *Lecanicillium fungicola* in the button mushroom, *Agaricus bisporus*,

assessed by Quantitative trait locus mapping. *Appl. Environ. Microbiol*., 2012b;**78**(7): 2435 – 2442.

16. Lin, F.C. Genetic correlations and path coefficient analysis of some important quantitative characteristics in *Lentinula edodes*. *Acta. Edulis. Fungi*., 1995; **2**(2): 9 – 12 (in Chinese)
17. Lin, F.X., Cheng, S.M., Pan, Y.J. Factor analysis for quantitative traits in *Lentinula edodes*. *Mycosystema*., 2004; **23**(4): 502 – 507 (in Chinese)
18. Lin, F.X., Cheng, S.M., Li, A.Z., Xu, X.F., Lin, F.C. Correlation and principal component analysis for major quantitative traits in *Lentinula edodes*. *Mycosystema*., 2006; **25**(4): 579 – 586 (in Chinese)
19. Santoyo, F., González, A.E., Terrón, M.C., Ramíreza, L., Pisabarro, A.G. Quantitative linkage mapping of lignin-degrading enzymatic activities in *Pleurotus ostreatus*. *Enzyme. Microb. Technol*., 2008; **43**(2): 137 – 143.
20. Li, A.Z., Xu, X.F., Lin, F.X., Chen, S.M., Lin, F.C. Cloning and identification of partial DNA fragment for *B* mating-type factor in *Lentinula edodes* using degenerate PCR. *World. J. Microbiol. Biotechnol*., 2007; **23**(3): 411 – 415.
21. Darmono, T.W., Burdsall, H.H. Morphological characteristics of incompatibility reactions and evidence for nuclear migration in *Armillaria mellea*. *Mycologia*., 1992; **84**(3): 367 – 375.
22. Olson, Å. Genetic linkage between growth rate and the intersterility genes S and P in the basidiomycete *Heterobasidion annosum s.lat. Mycol. Res*., 2006; **110**(8): 979 – 984.
23. Falconer, D.S. (ed): Introduction to quantitative genetics. Third. Longman Group Limited, London, 1989.
24. Van der Nest, M.A., Slippers, B., Steenkamp, E.T., De Vos, L., Van Zyl, K., Stenlid, J., Wingfield, M.J., Wingfield, B.D. Genetic linkage map for *Amylostereum areolatum* reveals an association between vegetative growth and sexual and self-recognition. *Fungal. Genet. Biol*., 2009; **46**(9): 632 – 641.
25. Larraya, L.M., Pérez, G., Iribarren, I., Blanco, J.A., Alfonso, M., Pisabarro, A.G., Ramirez, L. Relationships between monokaryotic growth rate and mating type in the edible basidiomycete *Pleurotus ostreatus*. *Appl. Environ. Microbiol*., 2001; **67**(8): 3385 – 3390.
26. Simchen, G. Monokaryotic variation and haploid selection in *Schizophyllum commune*. *Heredity*., 1966; **21**(2): 241 – 263.
27. Callac, P., Desmerger, C., Kerrigan, R.W., Imbernon, M. Conservation of genetic linkage with map expansion in distantly related crosses of *Agaricus bisporus*. *FEMS. Microbiol. Lett.,* 1997; **146**(2): 235 – 240.
28. Kerrigan, R.W.: A brief history of marker assisted selection in *Agaricus bisporus*. In: *Science and Cultivation of Edible Fungi* (Griensven V. ed). Rotterdam: Balkema, 2000; pp 183 – 189.