한 국 균 학 회 지 The Korean Journal of Mycology Research Article

Cultural and Morphological Characteristics of a New White Button Mushroom Cultivar 'Saedo'

Youn-Lee Oh[1,2]*, Kab-Yeul Jang[1], Won-Sik Kong[1], Pyung-Gyun Shin[1], Min ji Oh[1] and In-Geol Choi[2]

[1]*Mushroom Science Division, National Institute of Horticultural and Herbal Science, RDA, Eumseong 27709, Korea*
[2]*Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea*

ABSTRACT : Since development of the first Korean button mushroom cultivar 'Sae-Ah' in 2010, mushroom farmers have demanded an improved cultivar. Although a new cultivar, 'Saejeong', was developed in 2011, this cultivar has thus far been difficult to cultivate in typical mushroom farms. To solve this problem, another new cultivar, 'Saedo', was developed in 2012. This new cultivar was selected by genetic analysis from crossing the heterokaryon line A175 and the homokaryon line ASI1346-15. The heterokaryon A175 was a crossed line between strains ASI1038-211 and ASI1346-20. The mycelium of 'Saedo' showed good growth on compost dextrose agar at 25°C. The morphological traits such as the pileus (cap) and stipe of the 'Saedo' cultivar were thicker than those of the reference control 'Saejeong', and the production yield was increased by 1.89-fold compared to that of the reference control. A field experiment of the 'Saedo' cultivar has been ongoing since 2013. The yield of the 'Saedo' cultivar was found to be substantially higher than that of the reference control during the 2nd flush.

KEYWORDS : ***Agaricus bisporus*, Button mushroom cultivar, Cultural and morphological characteristics**

Introduction

Agaricus bisporus is one of the widely consumed mushrooms in the world because of good taste and flavor [1]. The production of *A. bisporus* has increased in China, the United States, Poland and the Netherlands [2]. In Korea, *A. bisporus* has been one of the most popular edible mushrooms and the total Korean production of *A. bisporus* was 11,493 MT (79 billion won) in 2014 [3]. The high productivity of valuable mushrooms in Korea was mainly attained by improving cultivation techniques developed from 1960s. Since the new cultivar 'Sae-Ah' for *A. bisporus* was developed in 2010, many trials were carried out to breed better cultivars.

A. bisporus has two haploids in the basdiospore as the multinucleate. This mushroom shows secondary homothallism that is capable of making a sexually reproducing colony from a single spore when it propagates in a complete isolation [4]. Whereas other mushrooms are heterothallism that reproduces sexually among individuals having compatible mating types [5]. Although *A. bisporus* mostly generates two spores, around 10~15% of basidia actually produce four spores having haploid nuclei that are compatible with the other haploid nuclei (homokaryons) [6]. Because of this low selection of spores based on homothallic nature, the breeding of *A. bisporus* is more difficult than that of other mushrooms. The usual methods for breeding of *A. bisporus* are mutation [7], mating [8-10], isolation and regeneration of protoplast [11], and transformation [12-14].

It is known that the mycelium of heterokaryons grows faster than putative homokaryons [15]. Because *A. bisporus* do not have clamp connections [16], putative homokaryons are distinguished from hetrokaryons by comparing the speed of mycelial growth of *A. bisporus*. This kind of selection was usually done for obtaining mother strains and checking the mating compatibility. Recently, molecular analyses such as restriction fragment length polymorphism (RFLP) [17], Random Amplification of Polymorphic DNA (RAPD) [18, 19] and Inter Simple Sequence Repeats (ISSR) [20, 21] are also used for selection of homokaryons instead of measuring mycelial growth.

Kor. J. Mycol. 2016 June, **44**(2): 94-99
http://dx.doi.org/10.4489/KJM.2016.44.2.94
pISSN 0253-651X • eISSN 2383-5249

***Corresponding author**
E-mail: o5ne2@korea.kr

Received May 30, 2016
Revised June 21, 2016
Accepted June 22, 2016

In order to develop improved cultivars giving high yield and good quality for farmers, breeders often utilize morphological and agronomic traits [22]. The cultural, morphological and agronomic traits of mushrooms are useful characteristics for the total yield and the quality of mushroom production. For instance, optimal temperature of mycelia growth and fruiting formation is known to affect the total yield. Also, when the cap color is whiter, the price of mushrooms is more expensive in the market.

The new cultivar 'Saedo' is the most popular cultivar in Korea. In this study, those morphological and agronomic characteristics of the new cultivar were investigated and compared to the reference control 'Saejeong' to classify two varieties and introduce the 'Saedo' cultivar.

Materials and Methods

Breeding procedure

We selected two strains, ASI 1038 and ASI 1346 among two hundreds of collections in mushroom department of National Institute Horticultural and Herbal Science. Over one thousands of single spore isolates (SSIs) were collected from spores of the selected two strains. SSIs were analyzed with a RAPD marker to select homokaryons (S1038-211, S1346-20). The S1038-211 and S1346-20 strains were crossed and resulted in a new crossed line (A175). A175 was crossed again with the homokaryon, S1346-15. From the second crossing, we developed a new cultivar, B397 that is designated as 'Saedo' (Fig. 1). The compatibility between homokaryons was identified by the RAPD analysis.

Measurement of cultural characteristics

The 'Saedo' cultivar cultured on CDA (4% dried compost, 0.7% Malt extract, 1% sucrose, 2% agar) media in various temperatures (15, 20, 25 and 30°C) for 14 days with three replicas. 'Sajeong' cultivar was used for the reference control. The mycelial growth was measured with vernier calipers on day 7 and 14 after inoculation.

Investigation of agronomic and morphological characteristics

The 'Saedo' and the reference control, 'Sajeong', were cultivated with three replicas to investigate morphological and agronomic characteristics at Mushroom Cultivation Center in mushroom department of National Institute Horticultural and Herbal Science, Eumseong, Korea. The mother spawns of two cultivars were made with wheat media (20 kg wheat, 0.6 kg gypsum, 0.3 kg calcium carbonate/100 bottles) and cultured for 14 days and cultured at 25°C, 65% humidity for 20 days. The 500 g of cultured spawns were inoculated in boxes (534 × 378 × 168 mm) with 10 kg phase II compost. The phase II compost was prepared at 65°C for 6 hrs and 55 for 7 days, respectively. The mycelium grows at 25, 80% humidity for 15 days. When approximately four fifths of the mycelium cultured in the compost, casing soil was put on the compost. Fifteen days after the casing soil was put, the growing temperature and moisture was changed into 16, 85% humidity to generate pin-heading. After harvesting mushrooms during the 1st and the 2nd flush, the total yield and the yield-related traits such as the number of the fruiting bodies and the mushroom weight was investigated [23]. As for the agronomic traits, the mycelia cultural period, the

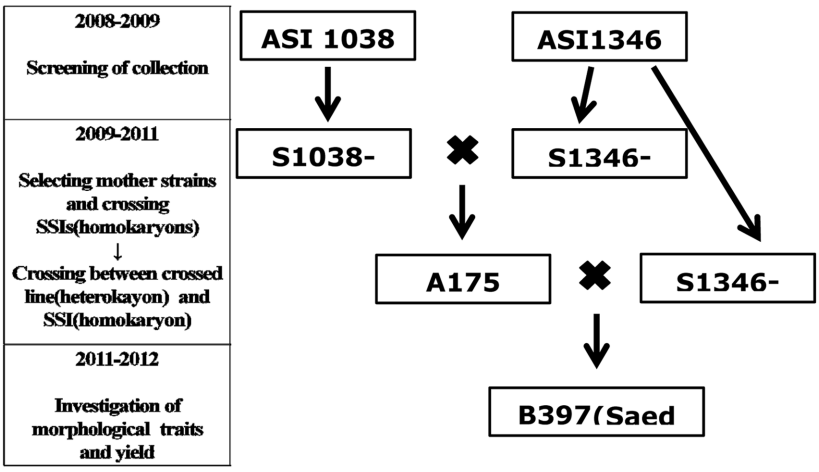

Fig. 1. Breeding pedigree of the new button mushroom, *Agaricus bisporus,* cultivar 'Saedo'.

earliness and the harvesting period were analyzed. Lastly, the diameter and the length of the pileus, the thickness and the length of the stipe, the whiteness (CR-400; Konica Minolta, Tokyo, Japan) and the hardness (TA1 Texture Analyzer; LLOYD, West Sussex, UK) of both the pileus and the stipe were measured as the morphological traits.

Field experiment

Two cultivars, 'Saedo' cultivar and 'Saejeong', were tested at eight mushroom farms in at Geoncheon-eup, Gyeonju-si in March 2015. The 'Saedo' cultivar was cultivated in two 215 m^2-scale farms and two 264 m^2-scale farms. The 'Saejeong' cultivar was cultivated in two 231 m^2-scale farms and two 264 m^2-scale farms. The yield was investigated from the 1st to the 5th flush and the fruiting bodies harvested in the 1st flush were measured in the diameter and length of the pileus, the thickness and the length of the stipe and the whiteness (CR-400; Konica Minolta) and the hardness (TA1 Texture Analyzer; LLOYD) of both the pileus and the stipe.

Results and Discussion

Breeding of 'Saedo' cultivar

The homokaryons have one meiotic nucleus from the tetrasporic basidia and thus give rise to self-sterile [24]. Appressed colony morphology and slow growth are morphological markers to identify homokaryons [25]. But it is time-consuming and difficult. Kavousi et al. [26] reported that homokaryons have significantly less bands than those of heterokaryons in RAPD analysis. In this study, over one thousands of SSIs were analyzed with a RAPD marker which is a selected marker from the OPA series (data not shown). The selected homokaryons from each strain (ASI 1038 and ASI 1346) were crossed. The compatibility of the crossed lines was confirmed by the selected RAPD marker, OPA12 primer (Fig. 2). Finally, the crossed line, B397, was selected as a superior line through investigation of productivity, agronomic and morphological traits.

Characteristics of mycelial growth

The mycelial growth was measured on MMP (2.09 g/L MOPS, 10 g/L malt extract, 5 g/L mycological peptone, 17.5 g/L agar, adjusted to pH 7.0 with KOH) agar [27] and CDA media in *A. bisporus*. In this study, we cultured on CDA media on day 7 and 14 at 15, 20, 25 and 30°C, respectively. The mycelial growth of the 'Saedo' cultivar was compared to the reference control, 'Saejeong'. In the early mycelia growth phase, the 'Saejeong' cultivar grew significantly faster than 'Saedo' at 15, 20 and 30°C, cultural temperature ($p < 0.05$), respectively. On day 14, the mycelium of the 'Saedo' cultivar grew as much as 'Saejeong' at 25°C (Fig. 3). In the mushroom farms the mycelium of spawn grows well when the compost temperature is maintained at 24~27°C [28], and thus it was identified that the 'Saedo' cultivar has an optimal mycelial growth in the farm.

Agronomic and morphological characteristics

The 'Saedo' cultivar and the reference control, 'Saejeong' were cultivated in 10 kg compost (per box) during the 1st and 2nd flush. The total production yield of the 'Saedo' cultivar, 937.4 (417.4) g, increased significantly with 1.89 folds more than the reference control, 496.1 (247.4) g. As for the morphological traits, the 'Saedo' cultivar was substantially thicker than the reference control in pileus, 26.6 (0.5) mm and stipe, 22.3 (1.2) mm (*t*-test, $p < 0.05$) (Table 1). Originally, *A. bisporus* has a pileus with broad, flat scales. But customers mostly see a hemispherical shape in

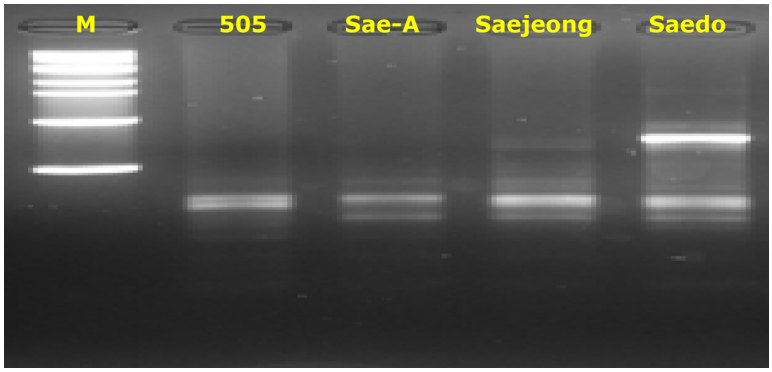

Fig. 2. RAPD fingerprints of new varieties in *Agaricus bisporus* with OPA12 primers (M: 1 kb markers) RAPD, random amplification of polymorphic DNA..

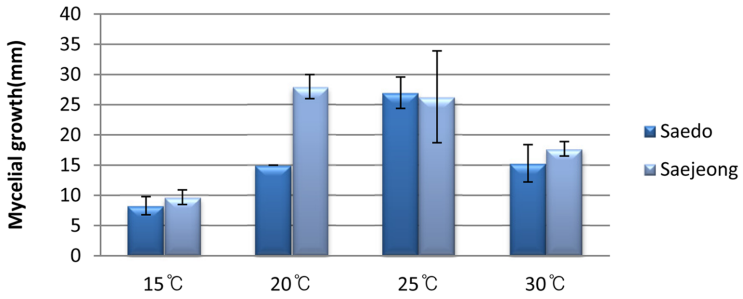

Fig. 3. Mycelial growth of two strains, Saedo and Saejeong (control), of *Agaricus bisporus* with three replicas at different temperatures after culture on day 14.

Table 1. Morphological and agronomic characteristics of the 'Saedo' cultivar and the reference control 'Saejeong' with three replicas at Mushroom Cultivation Center, Eumseong

	Lines	Saedo	Saejeong (Control)
Yield (g/10 kg compost)	Total yield*	937.4 (417.4)	496.1 (247.4)
	Mushroom weight	39.9 (4.5)	29.4 (2.3)
	Number of mushrooms	24.3 (10.5)	15.0 (5.0)
Agronomic traits (days)	Mycelial incubation period	22	22
	Earliness	19.0 (2.1)	21.0 (1.7)
Morphological traits (mm)	Diameter of Pileus	46.4 (0.3)	48.1 (0.6)
	Thickness of Pileus *	26.6 (0.5)	24.0 (1.8)
	Thickness of Stipe*	22.3 (1.2)	16.2 (4.6)
	Length of Stipe	23.6 (1.0)	26.5 (7.0)
	Hardness of Pileus	4.8 (0.3)	4.1 (1.1)
	Hardness of Stipe	5.2 (0.2)	5.1 (1.3)
	Whiteness of Pileus	92.0 (0.7)	90.7 (0.8)
	Whiteness of Stipe	86.9 (0.3)	81.0 (4.6)

Significant differences (t-test, $p < 0.05$ and < 0.01) between treatments for each time point are indicated by * and **.

supermarkets before it flattens out due to maturity [29]. From the mushroom's standpoint, the hemispherical shape is an early stage in their development, so the shelf life of a hemispherical shape mushroom is much longer than a broad shape mushroom. For this reason, the shape of the mushroom is important to its shelf life. That is why cultivars should have a large and thick pileus with a hemispherical shape and thick and short stipe to keep a long shelf life. With these findings, the 'Saedo' cultivar was identified as a cultivar with high productivity and good quality.

Field experiment

In this field experiment, the total production yield of the 'Saedo' cultivar, 12.8 (2.5) kg/m^2, was generally higher than the reference control, 'Saejeong' cultivar, 10.2 (3.3) kg/m^2. During the 2nd flush, the production yield of the 'Saedo' cultivar, 4.0 (0.8) kg/3.3 m^2 was considerably higher than the reference control, 2.76 (0.3) kg/3.3 m^2 (*t*-test, $p < 0.05$). During the 5th flush, only 'Saejeong' cultivated. Most farmers cultivate *A. bisporus* during the 1st until 3rd flush due to low productivity. As this point, 'Saedo' was proved as a high productive cultivar in average 10.1 kg/m^2 during the 1st and 2nd flush (Fig. 4). In morphological traits, the diameter of the pileus, 48.51 (2.2) mm, and the length of the stipe, 41.7 (4.0) mm, of the 'Saedo' cultivar was longer than the reference control, 42.6 (1.7), 35.5 (4.1) mm. The pileus, 27.3 (1.3) mm, and the stipe, 19.7 (2.0) mm, of the 'Saedo' cultivar is thicker than the reference control, 22.6 (2.0), 24.1 (5.5) mm (Fig. 5). But the hardness of the pileus of the 'Saedo' cultivar, 4.3 (0.6) N, was lower than the reference control, 5.7 (0.3) N. The

Table 2. Morphological characteristics of the 'Saedo' cultivar and the reference control 'Saejeong' in a field experiment in March 2015

	Lines	Saedo	Saejeong (Control)
Morphological traits (mm)	Diameter of Pileus**	48.5 (2.2)	42.6 (1.7)
	Thickness of Pileus**	27.3 (1.3)	22.6 (2.0)
	Thickness of Stipe*	19.7 (2.0)	24.1 (5.5)
	Length of Stipe*	41.7 (4.0)	35.5 (4.1)
	Hardness of Pileus**	4.3 (0.6)	5.7 (0.3)
	Hardness of Stipe	4.1 (0.3)	4.0 (1.0)
	Whiteness of Pileus	89.9 (1.8)	91.3 (0.7)
	Whiteness of Stipe	78.5 (5.4)	83.2 (9.4)

Significant differences (*t*-test, $p < 0.05$ and < 0.01) between treatments for each time point are indicated by * and **.

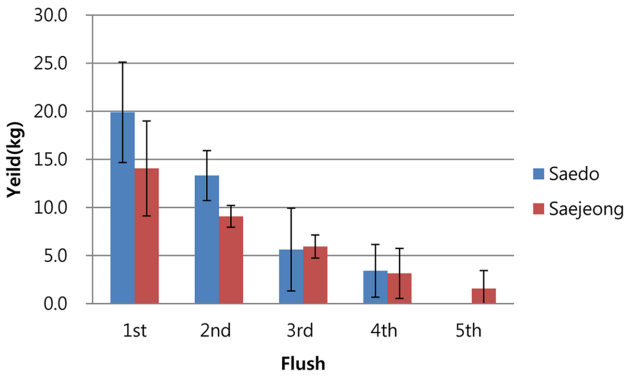

Fig. 4. The yield of the 'Saedo' cultivar and the control 'Saejoeng' on different flushes in a field experiment in March 2015.

Fig. 5. The outer part (A) and the inner part (B) of fruiting bodies of the 'Saedo' cultivar (right) and the reference control 'Saejeong' (left).

above morphological traits were statistically significantly different between the 'Saedo' cultivar and the 'Saejeong' cultivar (*t*-test, $p < 0.01$ or 0.05) (Table 2). From these results, we identified that the 'Saedo' cultivar had a better shape and higher productivity than the 'Saejeong' cultivar. The 'Saedo' cultivar occupies half of the distribution rate of Korean varieties, because of that reason. Also, 37% of the Korean farmers have cultivated these varieties last year.

Acknowledgements

This study was supported by a grant from the Golden Seed Project of 'Development of new variety to substitute imported one in button mushroom (*Agaricus bisporus*)' (213003-04-4-CGJ00), National Institute of Horticultural & Herbal Science, Republic of Korea.

REFERENCES

1. Geml J, David MG, Royse. Molecular evolution of *Agaricus species* based on ITS and LSU rDNA sequences. Mycol Prog 2004;3:157-76.
2. Sonenberg AS, Baars JJ, Hendrickx PM, Lavrijssen B, Gao W, Weijn A, Mes JJ. Breeding and strain protection in the button mushroom *Agaricus bisporus*. In: Proceedings of the 7th International Conference of the World Society for Mushroom Biology and Mushroom Products; 2011 Oct 4-7; Arcachon, France: Wageningen: ICMBMP; 2011. p. 7-15.
3. Ministry of Agriculture, Food and Rural Affairs. 2014.
4. Blakeslee AF. Sexual reproduction in the Mucorineae. Proc Am Acad Arts Sci 1904;40:205-319.

5. Ni N, Feretzaki M, Sun S, Wang X, Heitman J. Sex in fungi. Annu Rev Genet 2011:45:405-30.
6. Kerrigan RW, Baller LM, Horgen PA, Anderson JB. Strategies for the efficient recovery of *Agaricus bisporus* homokaryons. Mycologia 1992;84:575-9.
7. Pritsche G. Contribution to mutation research in *Agaricus bisporus*. In : Proceeding of the 6th International Mushroom conference; 1965 May 31-Jun 3; Wageningen and Amsterdam, Netherlands. Coatesville: ISMS; 1967. p. 27-47.
8. Elliott TJ. Comparative sexuality in *Agaricus* species. Microbiology 1978;107:113-22.
9. Kneebone LR, Shultz PG, Patton TG. Strain selection and development by means of mycelial anastomosis. In: Proceeding of the 8th International Mushroom conference; 1971; London, UK. Coatesville: ISMS; 1972. p. 19-25.
10. Moessner EJ. Preliminary studies of the possibility of obtaining improved cultures through mycelial fusion (Anastomoses). In: Proceeding of the 5th International Mushroom conference; 1962; Philadelphia, USA. Coatesville: ISMS; 1963. p. 197-203.
11. Horgen PA, Jin T, Anderson JB. The use of protoplast production, protoplast regeneration and restriction fragment length polymorphisms in developing a systematic and highly reproducible breeding strategy for *Agaricus bisporus*. In: Proceedings of the First International Seminar on Mushroom Science, Mushroom Experimental Station; 1991 May 14-17; Horst, Netherlands. Wageningen: Pudoc; 1991. p. 62-72.
12. van de Rhee MD, Graça PM, Huizing HJ, Mooibroek H. Transformation of the cultivated mushroom, *Agaricus bisporus*, to hygromycin B resistance. Mol Gen Genet 1996;250:252-8.
13. Chen X, Stone M, Schlagnhaufer C, Romaine CP. A fruiting body tissue method for efficient *Agrobacterium*-mediated transformation of *Agaricus bisporus*. Appl Environ Microbiol 2000;66:4510-3.
14. Mikosch TS, Lavrijssen B, Sonnenberg AS, van Griensven LJ. Transformation of the cultivated mushroom *Agaricus bisporus* (Lange) using T-DNA from *Agrobacterium tumefaciens*. Curr Genet 2001;39:35-9.
15. Yadav MC, Verma RN, Dhar BL. Studies on the development of improved strains and hybrids of white button mushroom *Agaricus bisporus* (Lange) Imbach. In: Proceeding of the 3rd international conference on mushroom biology and mushroom product; 1999 Oct 12-16; Sydney, Australia: Sydney: WSMBMP; 1999. p. 93-102.
16. Raper CA, Raper JR, Miller, RE. Genetic analysis of the life cycle of *Agaricus bisporus*. Mycologia 1972;64:1088-117.
17. Xu J, Kerrigan RW, Horgen PA, Anderson JB. Localization of the mating type gene in *Agaricus bisporus.* Appl Environ Microbiol 1993;59:3044-9.
18. Khush RS, Becker E, Wach M. DNA amplification polymorphisms of the cultivated mushroom *Agaricus bisporus*. Appl Environ Microbiol 1992;58:2971-7.
19. Kerrigan RW, Royer JC, Baller LM, Kohli Y, Horgen PA, Anderson JB. Meiotic behavior and linkage relationships in the secondarily homothallic fungus *Agaricus bisporus*. Genetics 1993;133:225-36.
20. Nazrul MI, Yin-Bing B. ISSR as new markers for identification of homokaryotic protoclones of *Agaricus bisporus.* Curr Microbiol 2010;60:92-8.
21. Nazrul MI, YinBing B. Differentiation of homokaryons and heterokaryons of *Agaricus bisporus* with inter-simple sequence repeat markers. Microbiol Res 2011;166:226-36.
22. Shortell RR, Meyer WA, Bonos SA. Classification and inheritance of morphological and agronomic characteristics in Kentucky Bluegrass (*Poa pratensis* L.). HortScience 2009;44:274-9.
23. Oh YL, Jang KY, Kong WS, Shin PG, Kim ES, Oh MJ, Choi IG. Development of a new brown button mushroom cultivar 'Hogam'. J Mushroom 2015;13:237-42.
24. Castle AJ, Horgen PA, Anderson JB. Crosses among homokaryons from commercial and wild-collected strains of the mushroom *Agaricus brunnescens* (=*A. bisporus*). Appl Environ Microbiol 1988;54:1643-8.
25. Sharma M, Suman BC, Gupta D. Characterization of single spore isolates of *Agaricus bisporus* (Lange) Imbach using conventional and molecular methods. Curr Microbiol 2014;69: 474-83.
26. Kavousi HR, Farsi M, Shahriari F. Comparison of random amplified polymorphic DNA markers and morphological characters in identification of homokaryon isolates of white button mushroom (*Agaricus bisporus*). Pak J Biol Sci 2008;11:1771-8.
27. Sonnenberg AS, Wessels JG, van Griensven LJ. An efficient protoplasting/regeneration system for *Agaricus bisporus* and *Agaricus bitorquis.* Curr Microbiol 1988;17:285-91.
28. Royse DJ, Beelman RB. Six steps to mushroom farming. 2nd ed. University Park: The Pennsylvania State University; 2007.
29. Zeitlmayr L. Wild mushrooms: an illustrated handbook. New York: Transatlantic Arts; 1976.