DNA FINGERPRINTING OF GENETIC DIVERSITY OF *AGARICUS BISPORUS*

ZE-SHENG WANG, MEI-YUAN CHEN, ZHI-XIN CAI, JIAN-HUA LIAO, HONG-RONG LI, ZHONG-JIE GUO, ZHENG-HUI LU

Edible Fungi Institute of Fujian Academy of Agricultural Sciences, Fujian Mushroom R&D Station

Fuzhou, Fujian, 350014, P.R.

China

mushroom@public.fz.fj.cn

ABSTRACT

In this study, 305 strains of *Agaricus bisporus*, including cultivars and wild strains, were studied by using the techniques of SRAP and ISSR fingerprinting, and 32 special SRAP bands, 28 special ISSR bands were obtained. These strains were clustered using the software NTSYSpc-2.02j based on the upper 60 special bands and a tree map was generated. Clustering results showed that the overall 305 strains can be divided into two major groups at the similarity coefficient value of 0.54.One group covered all of ARP strains, while another group included two main strains clusters, Chinese wild strains and cultivation strains. ARP strains were genetically distant from cultivars and Chinese wild strains. In cultivation strains group, the 206 cultivars can be divided into 7 sub-groups at the similarity coefficient of 0.9. While according to the agronomic characters of the strains, the 7 sub-groups belong to three groups: good-quality hybridized strains, good-quality non-hybridized strains and high-production strains. In Chinese wild strains group, 41 wild strains can be divided into three sub groups, which were geographically correlated, at the similarity coefficient value of 0.93. In this study, 120 strains in overall 305 strains can be identified through 60 special bands, which indicate the genetic diversity of germplasm resources of *A. bisporus*.

Keywords: *Agaricus bisporus*, SRAP, ISSR, cluster analysis

INTRODUCTION

Agaricus bisporus (J.E.Lange) Imbach is the most widely cultivated and consumed edible fungus in the world with important economic value. The cultivation of *A. bisporus* began in France 300 years ago, and its spawn purification, preparation and strain improvement has a history spanning over 100 years.

In about 1925, *A. bisporus* was introduced into China for cultivation. In 1978, the strain improvement started in China, and the technology of compost 2-phases fermentation was introduced into China by Prof. S. T. Chang, which greatly promoted the development of Chinese

button mushroom industry. In 1989, the hybrid strain As2796, which combined the characters of both high production and good quality, was bred by Fujian Mushroom R&D Station.

Since 1999, China had become the biggest country for *A. bisporus* production. The hybrid As2796 covered the largest cultivation area in China, and became one of the commercial strains producing largest annual yield of fresh button mushroom in the world. [1, 2, 3]

In 2007, Fujian Mushroom R&D Station was reorganized as one of the institutes of Fujian Academy of Agricultural Sciences, and established a germplasm resources library of *A. bisporus*, which preserved 435 cultivation or wild strains. It has become one of the most abundant germplasm resources conservation units of *A. bisporus* in the world.

SRAP (sequence-related amplified polymorphism) technology is a new type of molecular marker developed by Li *et al.* [4]. This molecular marker amplified by the primers which designed according to the open reading frames (ORFs) and can generate polymorphic amplification products owing to different individuals, intron numbers and length between promoter and intergenic regions. It has the advantage of simple, stable, well repeatability and is a very effective tool in genetic diversity analysis, cultivar identification and phylogenetic studies [5, 6, 7, 8, 9]

ISSR(Inter-Simple Sequence Repeat) is a kind of DNA markers developed based on microsatellites sequence [10] .It semi-randomly amplifies the area between SSR(Simple Sequence Repeat) employing artificially designed simple repeat nucleotide sequence as primers , and produces rich polymorphism. It has been used in a variety of economic crops on studying comparative genomics, genetic mapping, genetic diversity analysis, germplasm identification, and genetic variation analysis or phylogenetic and so on [10, 11, 12, 13, 14, 15, 8, 9].

In this study, two DNA molecular markers, SRAP and ISSR, were used to analysis the DNA fingerprint of 305 strains including the cultivation and wild strains of *A. bisporus* collected from around the world, in order to obtain their genetic relationship, and select a number of specific bands to provide the basis of DNA level for the identification of these strains, further more, selection of hybrid parents.

MATERIALS AND METHODS

Strains. 206 cultivation strains were collected from all over the world. 41 wild strains were collected from some places of Tibet, Xinjiang, Ningxia, Sichuan, Qinghai, Gansu province and so on in which no *A. bisporus* was cultivated and a good ecological environment was maintained. These strains have been preliminarily identified as wild *A. bisporus* by isozymes electrophoresis. 58 ARP strains were collected and shared by *Agaricus Resource Program* [16]. All the strains above were provided and preserved by the Edible Fungi Institute of Fujian Academy of Agricultural Sciences, P. R. China.

Reagents. PCR kits and other reagents were purchased from Shanghai Biological Engineering Service Co., Ltd. of China

SRAP and ISSR. Four hundred SRAP primer [Invitrogen Biotech (Shanghai) Co., China] combinations were screened in three representative samples from the 305 accessions. Under the

optimized condition, 3 primer combinations, generated strong and clear amplified bands, were selected for further investigations (Table 1). The SRAP reaction mixtures (total volume 20 μL) contained 60 ng DNA, 1×PCR buffer, 0.2mM dNTPs, 2mM $MgCl_2$, 1 unit Taq DNA polymerase and 0.4 mM of both forward and reverse primers. SRAP programmes involved an initial denaturation step of 5 min at 94℃, followed by 5 cycles of 94℃/1 min, 35 ℃/1 min, 72℃/1.5 min, 35 cycles of 94℃/1 min, 50℃/1 min, 72℃/1.5 min, and a final extension at 72℃/10 min.

A total of 80 ISSR primers [Invitrogen Biotech (Shanghai) Co., China] were screened initially with three representative samples. 2 primers that produced clear and reproducible fragments were selected for further analyses (Table 2). The ISSR reaction mixture (total volume 20 μL) contained 60 ng DNA, 1×PCR buffer, 0.5 mM primers, 0.2 mM dNTPs, 2mM $MgCl_2$, and 1 unit Taq DNA polymerase. The thermal cycling profile was: 94℃/5 min, followed by 45 cycles of 94℃/45s, 55℃/40s and 72℃/70s, and a final extension step at 72℃/10 min.

Table 1: SRAP primers combination and their sequences

me1- em2	me1: 5-TGAGTCCAAACCGGATA-3, em2:5-GACTGCGTACGAATTTGC-3
me2- em4	me2:5-TGAGTCCAAACCGGAGC-3, em4:5-GACTGCGTACGAATTTGA-3
me5- em10	me5: 5-TGAGTCCAAACCGGAAG-3, em10:5-GACTGCGTACGAATTTAG-3

Table 2: ISSR primers and their sequences

808	5-AGAGAGAGAGAGAGAGC-3
809	5-AGAGAGAGAGAGAGAGG-3

Method of mycelium cultivation, genome DNA extraction and SRAP and ISSR analysis. The method of mycelium cultivation, genome DNA extraction and SRAP and ISSR analysis were the same to the references [8].

RESULTS AND DISCUSSION

DNA fingerprinting analysis of 305 strains. Primer screen of SRAP and ISSR was carried out as described above, and 32 polymorphic bands of SRAP and 28 polymorphic bands of ISSR were got. Figure 1 to 5 shows part of SRAP and ISSR amplification patterns.

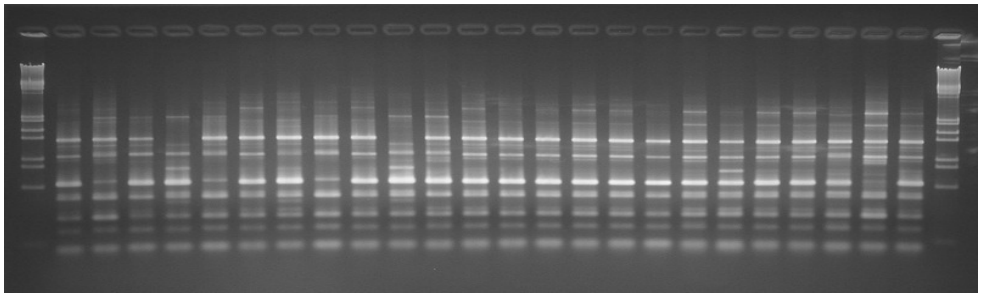

Figure 1: The SRAP patterns of 24 strains of *A. bisporus* (primers: me1-em2)
Note: Line M is Lambda DNA/EcoRI+HindIII Markers（Similar here in after）.

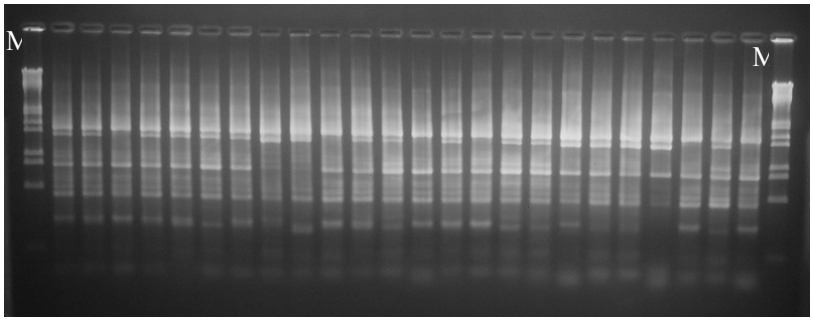

Figure 2: The SRAP patterns of 24 strains of *A. bisporus* (primers: me2-em4)

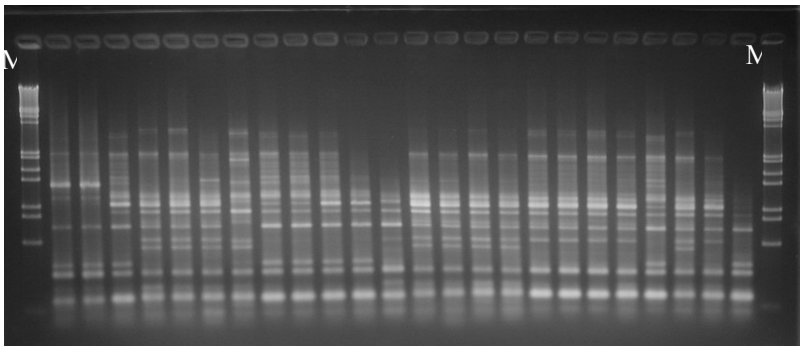

Figure 3: The SRAP patterns of 24 strains of *A. bisporus* (primers: me5-em10)

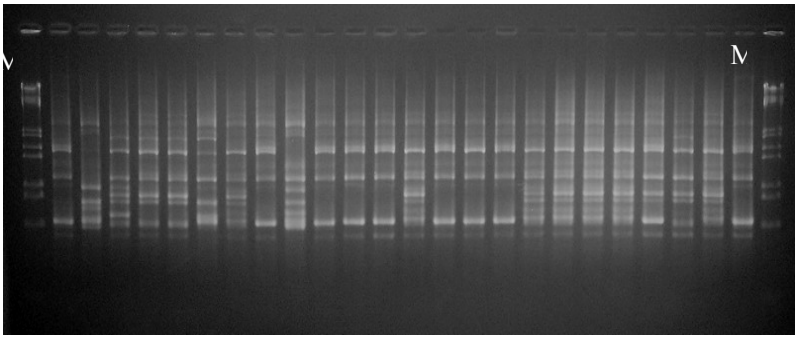

Figure 4: The ISSR patterns of 24 strains of *A. bisporus* (primer: 808)

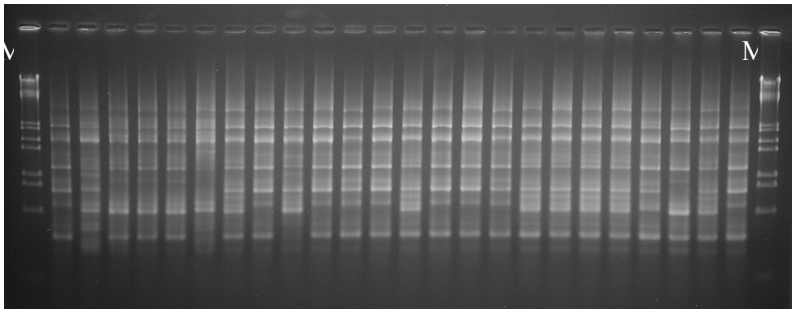

Figure 5: The ISSR patterns of 24 strains of *A. bisporus* (primer: 809)

Cluster analysis of 305 strains. Sixty specific bands obtained from SRAP and ISSR were converted into a (1, 0) data sheet (PCR positive recorded as "1", negative recorded as "0"), and clustered using NTSYSpc-2.02j software. Genetic relationship tree of overall 305 strains was

showed in Fig.6. Data showed that at the similarity coefficient of 0.54, these strains were classified into two major groups, one of which covered all of ARP strains, and another included Chinese wild strains and cultivation strains. The ARP strains completely separated from others, and were genetically distant from cultivars and Chinese wild strains. In cultivation strains group, the 206 cultivars can be divided into 7 sub-groups at the similarity coefficient of 0.9. While according to the agronomic characters of the strains, the 7 sub-groups belong to three groups: good-quality hybridized strains, good-quality non-hybridized strains and high-production strains. In Chinese wild strains group, 41 wild strains can be divided into three sub-groups, which were geographically correlated, at the similarity coefficient value of 0.93.

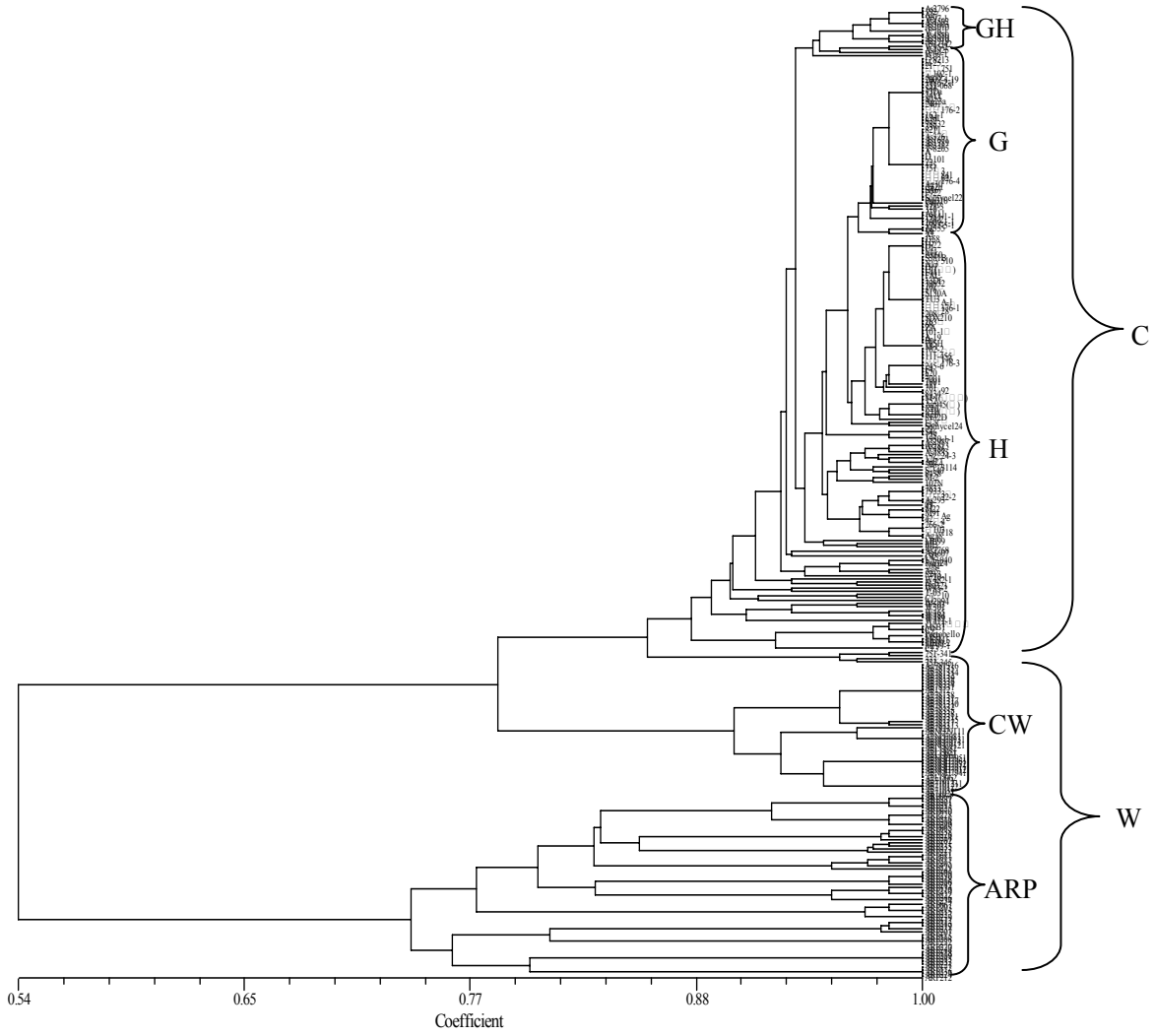

Figure 6: Clustering dendrogram of 305 strains of *A. bisporus* (W: Wild group, C: Cultivation group, H: High-production group, G: Good-quality non-hybridized group, GH: Good-quality hybridized group, CW: Chinese wild strains, ARP: ARP strains)

DISCUSSION

So far, rich germplasm resources of *A. bisporus* have been found. Since 1990s, many scientists worldwide carried out extensive research on the populations of *A. bisporus* about its distribution, behavior, community structure, gene flow dynamics and genetic variation [17, 18, 19, 20, 21, 22, 23, 24, 25]. *A. bisporus* germplasm resources were discovered one after another in Europe, America, Australia, Africa and Asia. In North America, Kerrigan had collected approximately 200 wild strains of *A. bisporus* through hosting ARP (*Agaricus Resource Program*) since 1988[16]. In Europe, a mushroom research group in France led by Callac, had collected about 250 wild strains of *A. bisporus* since 1990s [26]. In the UK, Elliott and Noble collected a lot of wild *A. bisporus* strains and also many strains of *Agaricus*. In China ,Wang *et al.* began to gather *A. bisporus* germplasm resources from all over the world since 1983[3], and had collected more than 400 strains of *A. bisporus* including 206 cultivars, 168 foreign wild strains and 61Chinese wild strains.

Genetic relationship research on *A. bisporus* has directive significance on strain identification, evaluation and breeding. Chen *et al*. carried out research on the molecular phylogenetic relationships about cultivars and wild strains respectively [8, 9]. In this article, 305 strains including cultivated and wild strains were studied together to explore their genetic relationship, and provide a scientific basis for the strain identification, evaluation and hybrid breeding parents selection. Clustering result showed that the genetic distance of wild and cultivated strains was large. Compared to cultivated strains, the PCR amplification patterns of wild strains (including Chinese wild strains and ARP strains) were more unique. In addition, 41 Chinese wild strains and 58 ARP strains clustered into individual group respectively, and separated from the cultivars. This may be caused by different source of strains. Wild strains tested are collected from China or America, while the cultivated strains were belong to European germplasm or their hybrids. The research also suggested that the populations of Chinese regional were genetically distant from European and American regional strains, which indicated that China has abundant germplasm resources of *A. bisporus* .These results were validated by isozyme electrophoresis identification (unpublished). So, isozyme electrophoresis identification is a feasible method in *A. bisporus* identification, and isozyme and DNA markers can be mutually confirmed in the identification of genetic relationships of *A. bisporus*. For the 206 cultivars, the strains with similar agronomic characters are mostly clustered together, indicating that the strains with similar agronomic properties also have relatively close genetic relationships.

CONCLUSION

Evaluation of germplasm of *A. bisporus* is very important for breeding. In this study, we used effective DNA fingerprinting tools to study the representative strains in germplasm resources library of *A. bisporus*. Results indicated that genetic distance of wild and cultivated strains was large. The genetic variation within ARP strains was in sharp contrast with the uniformity within the cultivars. This was consistent with previous studies [24, 27]. For wild strains (including Chinese wild strains and ARP strains), some of them has been confirmed that have a number of desirable traits not found in the present-day hybrids, i.e. temperature tolerance and disease resistance [28, 3]. So, full use of wild germplasm will be the key to breed new strains. For strain identification, through 5 primer pairs (combination), 60 specific markers screened in this paper,

120 species in 305 can be distinguished individually, while other strains were unable to be separated each other. This may be owing to their close genetic relationship, and more new markers or methods should be needed to identify them.

ACKNOWLEDGEMENT

We would like to thank Mark P. Wach and Kerrigan R.W for kindly providing us with ARP strains. This research supported by the earmarked fund for Modern Agro-industry Technology Research System, Nature Science Fund of China (30671456, 30771503), Nature Science Fund of Fujian Province (B0620004), Key S&T Program of Fujian Province (2006S1001, 2008SZ0002), and Key S&T Innovation Item of Fujian Academy of Agricultural Sciences (2007).

REFERENCES

[1]. Wang Z. S. et al. (1995). Studies on breeding hybrid strain As2796 of *Agaricus bisporus* for canning in China. *Mushroom Science* 14 (1): 71-72.

[2]. Wang Z.S. et al. (2001). Molecular genetic study on hybrid strain As2796 family of *Agaricus bisporus*. *Mycosystema*, 20(2), 233–237. (In Chinese)

[3]. Wang Z. S. et al. (2008). Guo ZJ, Study on the Biological Characteristics of Wild *Agaricus bisporus* Strains from China. *Mushroom Science*, XVII: 149-158.

[4]. Li G. & Quiros C. F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica, *Theor Appl Genet* 103: 455-461.

[5]. Budak H. et al. (2004). Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs and SRAPs. *Theor. Appl. Genet.* 109, 280–288.

[6]. Ferriol M. et al. (2003a). Genetic diversity of some accessions of *Cucurbita maxima* from Spain using RAPD and SRAP markers. *Genet. Res.Crop. Evol*. 50 (3), 227–238.

[7]. Ferriol M. et al. (2003b). Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theor. Appl. Genet*. 107, 271–282.

[8]. CHEN M.Y. et al. (2009a). DNA fingerprinting of diversity of *Agaricus bisporus* cultivation strains *J. Chinese Agricultural Science Bulletin*, 25(4):149-156.(in Chinese with English abstract)

[9]. CHEN M.Y. et al. (2009b). DNA fingerprinting of Ninety Wild Strains of *Agaricus* from China[J].*ACTA EDULIS FUNGI*, 16(1):11-16. (in Chinese with English abstract)

[10]. Zietkiewica E. et al. (1994).Genome fingerprinting by simple sequence repeat (SSR)–anchored polymerase chain reaction amplification. *Genomics* 20:176-183.

[11]. Khush R. S. et al. (1992). DNA Amplification polymorphisms of the cultivated mushroom *Agaricus bisporus*. *Appl.Environ.Microbial* 58:2971-2977.

[12]. PrevostA.& WilkinsonM. J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* 98,107–112.

[13]. Potter D. et al. (2002). Inter-simple sequence repeat markers for fingerprinting and determining genetic relationships of walunt (*Juglans regia*) cultivars. *J. Amer. Soc. Hort. Sci.* 127:75-81.

[14]. Pharmawati, M. et al. (2005). Molecular variation and fingerprinting of *Leucadendron*

cultivars (Proteaceae) by ISSR markers. *Ann. Bot.* 95, 1163–1170.
[15]. Muminovic J. et al. (2005). Genetic structure and diversity among radish varieties as inferred from AFLP and ISSR analyses. *J. Am. Soc. Hort. Sci*. 130 (1), 79–87.
[16]. Kerrigan R.W. (1996). Characteristics of a large collection of wild edible mushroom germ plasm: the *Agaricus Resource Program*. Pp 302–308 in: Culture collections to improve the quality of life, ed. by R. A. Sampson et al.Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
[17]. Callac P. et al. (1993). Morphological, genetic, and interfertility analyses reveal a novel, tetrasporic variety of *Agaricus bisporus* from the Sonoran desert of California. *Mycologia*, 85, 835–851.
[18]. Callac P. et al. (1998). Bsn-t allele from French field strains of *Agaricus bisporus*. *Applied Env. Microbiol*. 64:2105-2110.
[19]. Kerrigan R. W. (1995). Global genetic resources for *Agaricus* breeding and cultivation. *Can. J. Bot*. 73 (Suppl I):S973-S979.
[20]. Kerrigan R. W. et al. (1996). A summary of allelic diversity and geographical distribution at six allozyme loci of *Agaricus bisporus*. pp.57-66 in Royse D. J., ed. *Mushroom Biology and Mushroom Products*. The Pennsylvania State University, University Park, PA, USA.
[21]. Kerrigan R. W. et al. (1998). The coastal Californian population of *Agaricus bisporus* may be at risk of extinction. *Molecular Ecology*, 7:35–45.
[22]. Li G. et al. (2003). Gene for gene alignment between the Brassica and Arabidopsis genomes by direct transcriptome mapping. *Theor. Appl. Genet.* 107: 168-180
[23]. Sonnenberg A. S. M. et al. (1995). Detection of *Agaricus bisporus* viral dsRNAs in pure cultures, spawn, and spawn-run compost by RT-PCR. *Mushroom Science* 14:587-594.
[24]. Sonnenberg A. S. M. et al. (1999). Abr 1, a tranposon-like element in the genome of the cultivated mushroom *Agaricus bisporus* (Lange) Imbach. *Appl. Environ. Microbiol*. 65:3347-3353.
[25]. Wang H. C. & Wang Z. S. (1989).The prediction of strain characteristics of *Agaricus bisporus* by the application of isozyme electrophoresis. *Mushroom Science* 12(1):87-100.
[26]. Callac P. et al. (2000). Discovery of a wild Mediterranean population of *Agaricus bisporus*, and its usefulness for breeding work. *Mushroom Science* 15: 245-252.
[27]. Kerrigan R. W. (1995).Global genetic resource for *Agaricus* breeding and cultivation. *Can.J.Bot*.73:S973-S979
[28]. Dragt J. W. et al. (1995). Resistance in wild types of *Agaricus bisporus* to the mycoparasite *Verticillium fungicola var. fungicola*. *Mushroom Science* 14:679–683.