Mushroom Breeding Program In Iran

H. R. Gordan; M. Farsi**

** Industrial Fungi Biotechnology Research Group, ACECR-Mashhad Branch P. O. Box: 91775-1376,*
***Department of Agricultural Biotechnology of Ferdowsi University of Mashhad*
hrgordan@hotmail.com

Mushroom cultivation is a newly established, but growing industry in Iran. There are about 120 producers with a total of about 20,000 tones per year in the country. *Agaricus bisporus* consists of more than 85 percents of the total production of all mushrooms produced in Iran. Its yield average is about 12-15 kg/m^2, while in the global production it is about 27-30 kg/m^2. This is mainly due to using strains of genetically weak performance.

Since ten years ago a breeding program was started with emphasis on breeding high yielding strains in Mashhad. The short-term effort consisted of selection among single spore isolates and multispores cultures with a better performance in yield. The long-term effort consisted of employing of heterosis in hybrid strains. To reach the aim, more than 350 homokaryone isolates were selected through RAPD markers followed by yield trials from commercial and domestic strains, and crosses were made in many combinations using diallel method.

Selection among spores of commercial strains could somehow recover their potential genetic capacity, so that an average of 22 Kg/m^2 was recorded in the selected strains. Using growth type as a marker, it was possible to decrease the number of isolates in final stages of selection for homokaryones in solid medium or spawn. Cluster analysis based on average of band numbers emerged by RAPD markers, could separate homokaryotic and heterokaryotic isolates in two distinct groups. Some hybrids showed a better mycelia growth and a considerable higher yield than their parents. Efforts are now being made to collect wild strains of *Agaricus bisporus* in Iran and evaluating them for desirable genes including resistance to pest and diseases.

1. Introduction

Among the edible fungi, the white button mushroom, *Agaricus bisporus* (Lange) Imbach (= *A. brunnescence* Peck) holds a unique position, with a

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world wide production in large amounts. The economic value of *A. bisporus* in the late 1980s amounted 3.5 billion US dollars (Horgen *et al.,* 1992) while at the beginning of the new millennium has exceeded 10 billion US dollars. Now, the world production of this mushroom is near 38 percent of the world total production of edible mushrooms.

In spite of the economic and agronomic importance of *A. bisporus*, its breeding programs have always been problematic. Little information existed on the basic biology of this mushroom before 1970s. In the years 1970 and 1971 it was known that *A. bisporus* behaves as a secondarily homothallic (Miller, 1971, Elliott, 1972, Raper *et al.,* 1972), or more recently as an intramictic (Kerrigan, 1990) fungus during its life cycle. It means that each dikaryotic basidium cell performs a transient diploid followed by a meiosis division and production of four haploid meiotic nuclei. Majority of basidiospores receive two non-sister post meiotic nuclei and are therefore heteroallelic in over 90% of loci similar to the parents (Kerrigan *et al.,* 1993, Horgen *et al.,* 2002). The basidiospores germinate and rise to self-fertile heterokaryotic mycelium. Small percentage of basidiospores receives one of four post-meiotic nuclei and the smaller percentage receives two similar daughter nuclei. These two group of basidiospores rise to self- infertile homokaryotic mycelium (Kerrigan *et al.,* 1987, Khush *et al.,* 1995, Miles *et al.,* 1997). Thus, the homokaryons which are much important to the breeding programs are infrequent and this is an impediment to the button mushroom breeding (Horgen *et al.,* 1992, Khush *et al.,* 1995). In this species, there is no evidence to nuclear migration or fusion until fruit bodies are formed (Raper *et al.,* 1972, Kush *et al.,* 1995). In a heterokaryotic mycelium, each cross wall is multinucleate in which there are several copies of both non-sister haploid nuclei (Paul A. Horgen, Personal Communication). Homokaryons in *A. bisporus* and *A. bitorquis* are also multinucleate and each cross wall contains several copies of one haploid nucleus (Miles *et al.,* 1997). In these two species, the lack of clamp connections makes it difficult to distinguish heterokaryons (or dikaryons) from homokaryons and this is another impediment to their breeding (Chang *et al.,* 1989, Khush *et al.,* 1995, Loftus, *et al.,* 1995, Miles *et al.,* 1997). The other problem is that the basidiospore germination is a variable and slow process. There are also some problems with contamination in the germination particularly with bacteria, even under controlled laboratory conditions, so that bacteria colonize the medium before basidiospores can

germinate. Some germination kinetics of the basidiospores has been already studied (Horgen *et al.,* 1989, Kokorwicz *et al.,* 1994).

Strain improvement in *A. bisporus* has more been on the basis of selection and hybridization (Mehta *et al.,* 1994, Pandey *et al.,* 1994, Pathak *et al.,* 1998). Selection makes use of the variation which isolates perform in growth rate, colony type, yield and other characteristics, while hybridization often includes simple mixing and cross breeding (or strain hybridization). Simple mixing has not had a significant role in strain improvement of *A. bisporus* during its history of breeding. Cross breeding is often based on collecting the desired genes to novel strains in which the heterosis phenomenon will be probably observed. In this approach, crosses among compatible homokaryons, so-called anatomists, are needed (Castle *et al.,* 1988, Horgen *et al.,* 1992, Mehta *et al.,* 1994) and therefore, recovering the homokaryons is very important. Several traditional and modern molecular approaches are applied to recover and to confirm homokaryons (Castle *et al.,* 1988, Summerbell *et al.,* 1989, Horgen *et al.,* 1992, Kerrigan *et al.,* 1992, Khush *et al.,* 1995, Horgen *et al.,* 2002).

Mushroom cultivation is a newly established, but a fast growing industry in Iran. There are about 120 producers with a total of about 20,000 tones per year in the country. *Agaricus bisporus* consists of more than 85 percents of the total production of all mushrooms produced in Iran (The Iranian Mushroom Growers Association, 2004). Its yield average is about 12-15 kg/m^2, while in the global production it is about 27-30 kg/m^2. This is mainly due to using strains of genetically weak performance.

So far, no breeding program of *A. bisporus* had been conducted in Iran. Since ten years ago a breeding program was started with emphasis on breeding high yielding strains in Mashhad. The short-term effort consisted of selection among single spore isolates and multispores cultures with a better performance in yield. The long-term effort consisted of employing of heterosis in hybrid strains, using molecular markers to assist selections (Kerrigan, 2000).

The main objective of this program was to find approaches to produce high yielding strains with more adaptability to the country conditions. Thus, it was necessary to carry out a complex study to get a better understanding of the mushroom breeding and related problems. In this complex study, we attempted to find out the problems and to establish a framework for research on the *Agaricus bisporus* breeding in Iran.

Our new main objective is to collect wild strains of *Agaricus bisporus* in Iran and evaluating them for desirable genes including resistance to pest and diseases. Molecular tools will be also used in differentiating homokaryons from heterokaryons, evaluating strains and bred hybrids, and patenting the bred strains.

2. Materials and Methos

Basidiospore germination as the first step in a mushroom breeding program was first optimized by our lab conditions. Twenty days after transferring the single colonies, the mycelia of each single spore filled the medium enough and a pure isolate generated through a single spore (or a single spore isolate=SSI) was prepared. These cultures were used to inoculate the grains. Growth type and growth rate of colonies were measured on petri dishes. Investigations were made during 15 days after spore culture.

Spawn running was considered as a criterion for measuring variation among spawned beds. The observed yields were classified as follows: high, moderate, low and zero. Growth types (in solid medium and spawn) were classified as follows: strandy-fast, strandy-slow, fluffy-fast, fluffy-slow, appressed-slow and appressed-very slow. Then the relationship between the yield and the classes of growth type was measured. For study of some breeding characteristics, the variation of basidiocarps in terms of the ratio of cap diameter to stripe height, general and specific combining ability and different variations for several breeding traits were investigated. RAPD markers (Williams *et al.* 1990) were used to assist the selection of homokaryons, based on Khush's work (khush *et al.,* 1992). More than 350 homokaryotic isolates were also selected through RAPD markers. Cluster analysis (Ward method) was used to separate homokaryons and heterokaryones based on RAPD bands and to select furthest isolates for hybrids parents.

3. Results

The rate of generating single colonies between days 5-12 was more than other days. The rate of spore germination in PDA medium was more than that

of the CYM medium ($p \leq \%1$). Generally, after twelve days, the colony of each single spore could be observed with naked eyes. At this time, it was possible to transfer the single colonies to a fresh medium. Fifteen days after spore culture, the colonies of basidiospores grew together well and filled the medium.

A considerable variation was observed among SSIs. They varied in colony type and growth rate. Four classes of colony types including strandy, fluffy, cottony and appressed were observed. The observed growth types included fast slow and very slow. Most SSIs with a fast or slow growth in solid medium had a corresponding spawn running in the bed. The light microscopic observations also showed the different features of mycelia related to different growth type (Li *et. al.,* 1994 and Heath *et al.,* 1995).

Heterokaryons and homokaryons were distributed in all classes of growth types and were overlapped. However, selection based on growth type, lowered the number of isolates involved in the final stages of breeding program, So that the frequency of desirable homokaryons increased in final stage before going to yield trials. Then isolates that meet the requirements (a high yield or a homokaryotic status) were used in yield trials for further investigations.

Fourteen days after inoculation, considerable variations were observed among spawns. In each SSI, the observed colony type and growth rate in solid medium and spawn was the same ($p \leq \%1$).

Not a significant correlation was found between growth types such as slow or very slow growing appressed isolates, and their yields. However, most of them had a low or zero yields. There was also not found any significant relationship between growth types such as fast growing strandy or fluffy isolates, and yield, but the majority of such isolates produced a high yield, while the minority of them had a low yield. Therefore, it is possible to decrease the number of isolates involved in selection in the stage of solid medium or spawn based on growth type. It looks like the most effective approach is both excluding of very slow growing appressed isolates and retaining of fast growing strandy ones in the stage of solid medium.

Selection of isolates that did not produce any mushrooms (i.e. homokaryons) is also challengeable. Based on this study, omitting of fast growing strandy and fluffy isolates (which most of them produce a considerable mushroom) and selection of slow or very slow growing isolates (which most of them produce no mushroom) increased the efficiency of screening of homokaryons (Kerrigan *et al.,* 1992).

In this study, in addition to single spore isolates, multi spore cultures were also used. In any multi spore culture, several growth types were observed. Samples obtained from these cultures showed also different growth types. However, all classes of growth types were not observed similar to SSIs and also there was not a significant relationship between growth types and yield. But selection based on multi spore culture can have an effective influence upon strain improvement in *Agaricus bisporus*, because several growth types could be observed simultaneously in one petri dish.

Some hybrids obtained from this study, showed a higher performance than their parents and showed a considerable high yield in further fruiting tests (22 kg/m^2).

Here it should be stated that the fruiting test is often necessary for final verifying of any breeding program (Paul A. Horgen, Personal Communication). However, some homokaryons produced a few mushrooms and also some heterokaroyns produced no or a few mushrooms, due to the effect of some environmental conditions or other unknown factors (Kerrigan *et al.,* 1992) and this makes the matter more complex.

Cluster analysis based on average of band numbers emerged by RAPD markers, could separate homokaryotic and heterokaryotic isolates in two distinct groups.

Use of RAPD markers in the breeding programs of *A.bisporus,* was first reported by Khush *et al*. (Khush *et al.,* 1991, 1992, 1995). By having two different types of nuclei, heterokaryons have more sites for annealing of primers and consequently more segments of DNA can be amplified. Conversely, homokaryons have one type of nucleus and thus have fewer sites for annealing of primers. In this study, RAPD markers showed that they are able to distinguish homokaryon from heterokaryon and also high yielding isolated from the others. Efforts are now being made to collect wild strains of *Agaricus bisporus* in Iran and evaluating them for desirable genes including resistance to pest and diseases.

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