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RESEARCH ARTICLE

A comparative study of agronomic characteristics in several Iranian wild isolates of the button mushroom, *Agaricus bisporus*

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Abstract

The white button mushroom, *Agaricus bisporus*, is the most common edible mushroom cultivated in the country, which is very attractive among mushroom consumers. However, all the strains of *A. bisporus* are commercially imported and no research work has been done on wild populations of this mushroom. The aim of this study was, thus, to evaluate qualitative and quantitative traits of six internal transcribed spacer-authenticated indigenous strains, compared with two commercially cultivated strains of *A. bisporus* that served as controls. Cultivation experiments were conducted in a mushroom farm using a randomized complete block design with eight strains and three replications. During the picking period, most of the wild isolates exhibited relatively good quality traits in comparison with the controls. However, wild isolate As005 was the only wild isolate to yield mushrooms with an average of 45 mm cap, similar to those of commercial strain H737 and significantly larger than the rest ($p < 0.05$). In addition, while the cap color of all the wild mushrooms was brown, As005 showed a very light brown color. The yields of the tested wild isolated showed that As005 had a more productivity in the first and second flushes compared to the rest of wild isolates ($p < 0.05$). Overall, the results showed that As005 had quality and yield characteristics very similar to those of the controls. In conclusion, As005 may be considered as a good candidate in further *A. bisporus* breeding programs.

INTRODUCTION

The white button mushroom, *Agaricus bisporus*, is the most important economic and commercial mushroom that is widely cultivated in most countries of the world. Currently, there are more than 70 varieties of *A. bisporus;* major ones include: crimini mushroom, baby portobello, baby bella, mini bella, portabellini, Roman mushroom, Italian mushroom, and brown mushroom. While the diversity of gene pool is an important prerequisite for any breeding (Singh and Kamal, 2011), the commercial cultivars of *A. bisporus* are unable to bear a broad genetic diversity, which is mainly due to unusual life cycle of *A. bisporus* as a secondary homothallic fungus (Royse and May 1982). Although *A. bisporus* is not easy to manipulate through breeding, there are several approaches to improve performance of this mushroom, including strain selection based on single or multi-spore culture, intra-species hybridization, genetic transformation (Chakravarty, 2011) and introgression of desirable genes from wild germplasms into genetic background of this species (Geml and Royse, 2002). Todays, exploitation of wild genetic resources to diversify genetic background is considered a promising tool to develop new varieties of *A. bisporus* (Foulongne-Oriol, 2011).

There are several morphological, physiological and agronomic traits in the button mushroom that could be targets for a breeding program. Some of the important morphological characteristics include: stalk length, and cap diameter. These traits directly attribute to quality of mushrooms and improve marketability. They could also be considered as agronomic traits, as they play a role in total yield. Physiological characteristics consist of protein materials, secondary metabolites, disease resistance and nutritional value (Geml and Royse, 2002). Significant agronomic traits usually include total yield, number of flushes, and yield of each flush.

The white button mushroom accounts for more than 90% of the total mushroom production In Iran. However, there is a low genetic diversity among the commercial strains of this mushroom. In Iran, there are various regions in which *A. bisporus* has been reported to grow wild. However, to the best of our knowledge, no study has reported use of the Iranian wild populations of *A. bisporus* in mushroom breeding programs. Following collection and identification of a number of wild isolates of *A. bisporus* from Iran, this study was carried out to investigate agronomic characteristics of several wild isolates of *A. bisporus*, as compared to commercial cultivars of this mushroom in a commercial mushroom farm. Findings of this study would be an initial step towards using the broad genetic diversity of wild populations of *A. bisporus* in mushroom breeding programs.

Materials and methods

Strain preparation

The wild isolates of *A. bisporus* evaluated in this study have been collected by us from various regions of the north-east of Iran during an effort to collect wild *Agaricus* spp. mushrooms in the year 2012. These samples have previously been authenticated through internal transcribed spacer (ITS) sequence analysis, as described elsewhere (data not published yet). The wild isolates have been designed as As001, As003, As004, As005, As007, and As0011. In addition to the wild samples, two commercially cultivated strains of *A. bisporus* were used to serve as controls: H737 and IM008. All the mushroom samples were prepared as pure mycelial cultures from the mushroom bio-bank of Industrial Fungi Biotechnology Research Department, ACECR-Mashhad Branch, Iran. The samples were further sub-cultured in freshly prepared compost extract agar (CEA) media. In brief, 100 g of phase II compost was boiled for 20 minutes followed by several stages of filtration. The compost extract was then mixed with 1% agar (Merck, Darmstadt, Germany), v/w. The tissue culture was performed using the leading edges of mushroom mycelia and placed on the middle of the fresh media under aseptic conditions. The plates were incubated in 25°C until they were seen to grow properly in the media.

Spawn Preparation

The mycelial cultures of the mushrooms were subjected to spawn production using the wheat grains supplemented with conditioning agents including gypsum and lime. The spawn bags were incubated in 25°C until they were fully grown.

Cultivation experiments

Mushroom growing was performed based on standard cultivation methods for *A. bisporus* in a commercial mushroom farm. Compost preparation, casing, and aeration were performed as part of routine operations of the farm. Each compost block weighed approximately 16 kg. Mycelium growth through the compost and casing layer was completed at temperature 25 ° C and 90% humidity. Casing was done using a proper pasteurized soil about 4 cm thick as described by Maheshwari (2013). Aeration was done after completion of mycelia growth in the casing layer, along with a reduction in temperature (2-3ºC per day to 19ºC). Evaluation of quality and yield traits of fruiting bodies was performed when the veils were still closed (the membrane on the underside of the cap was still tightly attached to the stalk and cap)

Statistical analysis

The cultivation experiments were carried out based on a randomized complete block design with three replications. Data analysis was performed using the software SAS version 9.1. Duncan's test was utilized to compare treatments (mushroom strains) means. The correlation coefficients were generated using the same software. Traits evaluated in this study were grouped into two categories: qualitative and quantitative traits. Quality traits included: spawn growth, stalk length, cap color, and cap diameter. Quantitative traits included the yields of first and second flushes.

Results and Discussion

Spawn growth

The results showed that the average period of spawn bags filling was 22 days for all the strains; without any statistical differences observed between the strains ($p > 0.05$). These observations led to the postulation that the wild isolates of *A. bisporus* had a high potency of spawn making at the same rate as that of the commercial strains (Table 1). Spawn growth is a quality and measurable characteristic of mushroom strains. A fresh and active spawn plays

an impotant role in successful production of any mushrooms (Pani, 2011). In addition, the period of substrate colonization by a mushroom strain is important, as a long time to complete spawn may result in increasing the chance of infection of spawn. Many factors can determine the proper
spawn-growing period (Royse,1982) . Our observations with spawn growth of the testes mushroom strains might suggests that the wild isolates can be utilized to produce spawn commercially in a short period of time.

Cap color

Cap in *A. bisporus* mushrooms is easily recognizable with a white, cream or brown color. In this study, there were differences in the cap color among the tested strains. The colors evaluated in this study were constant from the early stages of the pin-heads to the stage of maturity. The cap color in the commercial strains H737 and IM008 was white, whereas the wild isolates generated brown capped mushrooms. However, As005 showed an apparent light brown color as compared to the rest of other wild isolates (Table 1). In this study, special care was taken in order to differentiate between the brown color caused by irrigation or bacterial infection and the inherited cap color of the strains.

As opposed to many other countries, there are limited cultivars of *A. bisporus* in Iran. As a result, the white button mushroom is the most common mushroom in the market. Thus, a brown capped mushroom is less likely to capture the consumer's attention in the current mushroom market, as compared to the white mushrooms. Among the wild isolates, the strain As005 with a light brown (compared to the other wild isolates with a dark brown) could be more valuable for both the mushroom market and mushroom breeding programs.

Stalk length

The *A. bisporus* stalk is central, circular (cylindrical) and firm. Stalk lengths of the mushrooms strains investigated in this study varied significantly. The strains As001, As003, As004, As005, and As0011 were in one group, with the shortest stalk that was significantly different from that of commercial strain H737 ($p < 0.05$). In addition, the stalks of wild isolates As003 and As004 were significantly shorter than those of both commercial strains ($p < 0.05$). The longest stalks belonged to strain As007 (ca.. 22 mm) and then H737 (ca 32 mm) (Table 1).

The stalk lengths of the wild isolates tested in this study were much lower than those reported with a commercial strain of *A. bisporus* reported previously (Demirer et al., 2005). Stalk lengths of *A. bisporus* mushrooms are directly used to classify their quality (Demirer et al., 2005). Variations in stalk lengths could be genetically determined or might be a response to cultural influences (Kerrigan and Wach, 2008). It should be taken into account that the absolute stalk length is commercially and economically important. Longer stalks in combination with a tightly closed cap might positively increase total yield and facilitate mechanical harvesting (Kerrigan and Wach, 2008). In accordance with this, we noticed a strong correlation (ca 69%) between stalk length and the weights of first and second flushes. However, in some mushroom markets, including Iran, short stalk mushrooms are more desirable and have higher marketability as compared to long ones. Parts of the stalk are usually cut in some countries to feed animals; but in Iran it might be infected with pesticides and should be discharged and cannot be used. Thus, a long mushroom stalk may not necessarily be considered a good quality and economically important character. Our observations also showed that the short stalk mushrooms had a tighter closed cap; thus their quality (at least in the Iranian mushroom market) should be considered higher as compared to the rest. Thus, except the wild strain As007, the other wild strains exhibited a good quality of stalk length, as compared to H737.

Cap diameter

The results showed that As005 and H737 were both in one group with an average of 45 mm cap width, which was significantly larger than the rest ($p < 0.05$). The next group was composed of three members As001, As007, As0011 and IM008 followed by As003 and As004. The minimum cap diameter was observed for As004 with about 17 mm cap width (Table 1).

Except As005 and H737, the cap diameter values observed in this study were lower than those reported with a commercial strain of *A. bisporus* (Demirer et al., 2005). The cap is the preferred part of the button mushroom; thus, mushrooms with high cap weights and low stalk weights are desirable (Demirer et al., 2005). Marketability, performance and total yield of the button mushroom is affected by the cap diameter. Accordingly, we noticed that the diameter of the cap significantly and positively correlated to the weight of both the first and second flush (each about 67%). The provisions of the Commission Regulation of the European Community (binding since 1 January 2003) states that size of cultivated mushrooms is determined by the maximum cap diameter and the stalk length (Demirer et al., 2005). Accordingly, the minimum cap diameter must be at least 15 mm for closed, veiled and open mushrooms (Demirer et al., 2005). In this study, all the values obtained with cap diameters were higher than this limit, particularly the wild strain As005 showed an outstanding cap width that was not significantly different from the commercial strain H737.

Yield of the first flush

This quantitative trait is directly related to total yield and significantly can affect the market value of the picked mushrooms. Among the wild isolates, As005 showed the highest amounts of yield in the first flush, that was not significantly different from commercial strain IM008 ($p > 0.05$). The commercial strain IM008 strain ranked the second followed by the other wild isolates As001, As003, As004, As007, and As0011 (Figure 1).

Yield of the second flush

The commercial strains H737 and IM008 exhibited the highest amounts of yield in the second flush, which was significantly different from the rest ($p < 0.05$) followed by As005 strain. The rest of the wild strains did not show significant difference among themselves (Figure 2).

In terms of mushroom growers, yield of second flush is considered more important than the rest of flushes, as the total yield reaches a pick in the second flush. In this study, the yield of the first flush strongly correlated (over 99%) with that of the second flush.

The yield percentage is usually expressed by dividing the weight of the second flush on the weight of compost blocks and the resulting value is multiplied by 100. Mushroom growers prefer a yield percentage between 18 to 22%. In this study it was observed that commercial strain H737 had a yield percentage of 18%. Among the wild isolates, As005 had the highest percentage (15.5%) that was similar to that of another commercial strain IM008 (16.5%). The yield percentages of other wild isolates were 12% on average.

Table 1. Assessment of quality traits among several wild and commercial isolates of *A. bisporus*

Strain	Spawn growth (day)	Cap color	Stalk length (cm)	Cap diameter (cm)
H737	21±2[a]	White	3.167[a]	4.5[a]
IM008	22±2[a]	White	1.916[bc]	2.83[b]
AS003	22±2[a]	Dark brown	1.5[d]	2.083[cd]
AS004	22±2[a]	Dark brown	1.5[d]	1.75[d]
AS007	23±1[a]	Dark brown	2.167[b]	3.167[b]
As001	23±1[a]	Dark brown	1.67[cd]	2.583[bc]
AS005	23±1[a]	Light brown	1.83[bcd]	4.5[a]
AS0011	24±1[a]	Dark brown	1.67[cd]	2.83[b]

Values represent mean±SD derived from three independent experiments. Means that do not share a letter are significantly different ($p<0.05$).

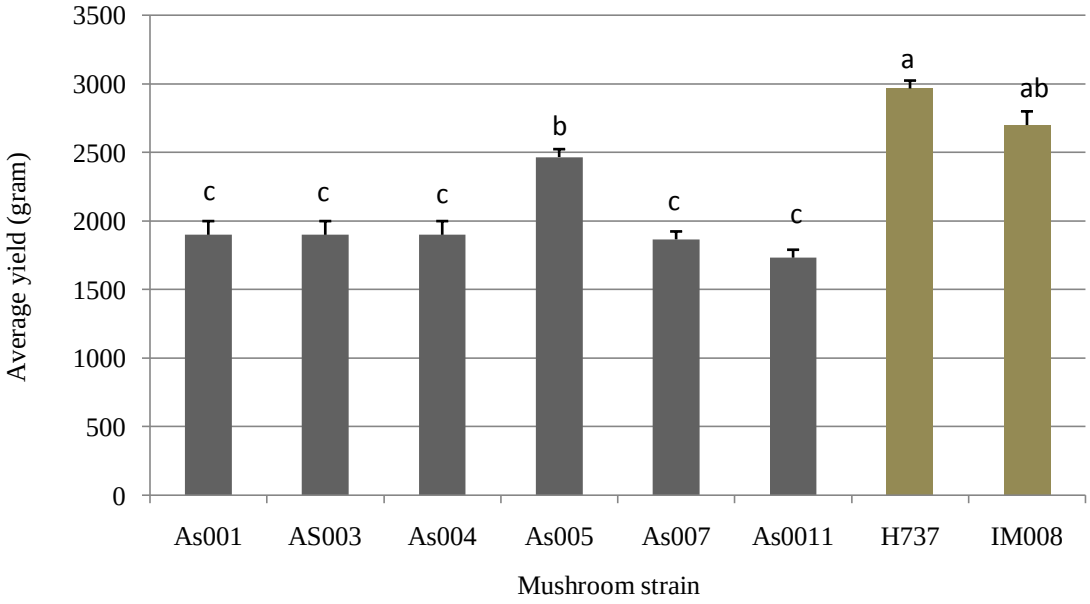

Figure 1. Yields of various wild and commercial *A. bisporus* during the first flush. Values (in gram) represent mean ± SD derived from three independent experiments. Each value shows the mushroom yield of a 16-kg compost block. Cultivation experiments were conducted in a mushroom farm using a randomized complete block design with eight strains and three replications. Measurement of mushroom yield was performed when the veils were still closed. Means that do not share a letter are significantly different (p<0.05).

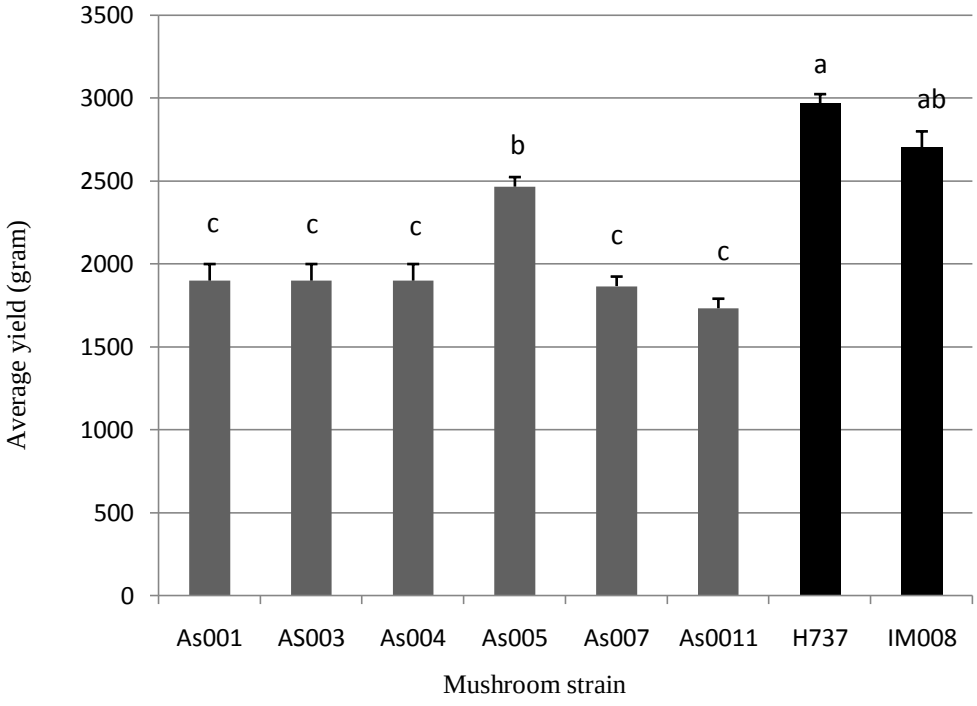

Figure 2. Yields of various wild and commercial *A. bisporus* during the second flush. Values (in gram) represent mean ± SD derived from three independent experiments. Each value shows the mushroom yield of a 16-kg compost block. Cultivation experiments were conducted as described in Fig. 1. Means that do not share a letter are significantly different (p<0.05).

Conclusion

Various quality and yield characteristics of several Iranian wild isolates of *A. bisporus* were investigated. In addition, two commercially cultivated strains of the white button mushroom were used as positive controls. This was the first report on evaluating agronomic characteristics of Iranian ITS-authenticated wild isolates of *A. bisporus* in a commercial mushroom farm. Overall, the findings revealed a good potential for wild isolate As005 that exhibited outstanding characteristics. This candidate isolate could potentially be used for further *A. bisporus* breeding programs. However, questions remain to be investigated in further studies, including whether the yield of As005 would show a stable performance in several mushroom farms over a specific period of time. In addition, a wider range of quantitative and quality of characters should be taken into account, including resistance to diseases. The wild mushrooms used in this study may not be directly used in the diet before making sure they are as safe as commercially cultivate strains of *A. bisporus*. The brown cultivars of *A. bisporus* are usually firm and have a good shelf life. However, the brown color of these wild isolates would negatively affect their marketability, at least in Iran. Thus, if the market demands a white color, the brown isolates tested in this study need to be crossed with the appropriate white cultivars of *A. bisporus* in order to select for white capped mushrooms.

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Declaration of interest There is no conflict of interest.

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