Trends in Mushroom cultivation and breeding

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Abstract

Mushrooms are highly nutritious and environment friendly crops that carry numerous medicinal benefits. The cultivation of edible mushrooms carries great relevance in today’s world in the context of a burgeoning population growth and extreme pressure on the environment. But advances in research on mushroom breeding and production is very limited compared to other crops. This maybe partly due to a lack of previous knowledge of the genetics and breeding system in this crop. Classical breeding in mushrooms has been difficult due to the predominantly secondary homothalic life cycle of this fungus. The cultivated strains thus display limited genetic variability. Also, developing an efficient genetic transformation technique and disease resistance is a challenging task in mushrooms. With the ongoing sequencing of the mushroom genome, knowledge of the gene organization and functions can be available and will help in developing better marker assisted selection breeding systems. This will lead to superior strains and along with an improvement in cultivation techniques, will pave the way for higher yield and quality.

Keywords: Mushrooms, Cultivation, Breeding system, Disease resistance, Transgenic breeding.

Abbreviations: MAS- Marker assisted selection; RAPD- Random amplification of polymorphic DNA; QTL- Quantitative trait loci.

Introduction

Mushrooms have been cultivated since ancient times for their nutritional value and flavour especially in the far eastern countries. The protein found in mushrooms is less than in animals but much more than in most plants. They have low fat content, high fibre and all essential amino acids and with the exception of iron, contain all important minerals too (Sadler, 2003). On exposure to UV-light, mushrooms also produce large amounts of vitamin D, which is normally difficult to obtain from a regular diet intake. In light of the growing incidences of cancer in today’s world, it is high time people woke up to the beneficial effects of mushrooms and utilized their cancer-fighting qualities. This low cost vegetable is not only packed with nutrients like vitamin D but also has properties to ward off cancer, HIV-1 AIDS and numerous other diseases (Beelman et al., 2003). It is an economical crop to cultivate, requiring low resources and area, can be grown throughout the world and all over the year from low-cost starting materials. There is tremendous potential and appeal for growing a highly nutritious food with excellent taste from substrates that are plentiful and not very expensive (Beetz and Kustudia, 2004). Also, it is very environmental friendly, capable of converting the lignocellulosic waste materials into food, feed and fertilizers (Hadar et al., 1992; Jaradat, 2010). However, mushroom consumption and production is relatively low in comparison to other crops and investment in the mushroom industry is still small compared to other crops and therefore investment is limited.

Common cultivated mushrooms

Although there are well over 300 genera of mushrooms and related fleshy basidiomycetes, only a few species of these fungi are cultivated commercially. This maybe due to the fact that many of them are mycorhizal and may not sporulate in the absence of the host. But many saprophytic species have been amenable to cultivation. Some of the more common cultivated species listed here (Table 1) are the button mushroom, Agaricus bisporus which was widely cultivated in Europe before being exported to North America by the settlers; the Shiitake mushroom (Lentinus edodes) which is grown for centuries in China and other oriental countries and the oyster mushroom (Pleurotus ostreatus) which was collected as wild specimens from forests in Florida and later actively cultivated in several countries around the world. Also grown are the oriental Enoke or velvet stem mushroom (Flammulina velutipes) whose major production is in Japan; the paddy straw mushroom (Volvariella volvacea) and ear fungus (Auricularia auricula) which has great medicinal value. Other cultivated mushrooms are the Reishi mushroom (Ganoderma lucidum) which is used as an alternative medicine and also as flavouring agent in Japan; the Nameko (Pholiota nameko) grown in the orient and Tremelia fuciformis or white jelly fungi that is grown for use as food supplements in Taiwan. Varieties of A. bisporus that are grown commercially include the crimini and portabella. Truffles (Tuber species) live in close mycorhizal association with roots of specific trees. They are considered a food delicacy and rated as one of the most expensive natural food in the world (Trappe et al., 2007).
<table>
<thead>
<tr>
<th>Common name</th>
<th>Latin name</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Button mushroom</td>
<td><em>Agaricus bisporus</em></td>
<td>Europe</td>
</tr>
<tr>
<td>Shiitake mushroom</td>
<td><em>Lentinus edodes</em></td>
<td>China</td>
</tr>
<tr>
<td>Oyster mushroom</td>
<td><em>Pleurotus ostreatus</em></td>
<td>Florida</td>
</tr>
<tr>
<td>Velvet stem mushroom</td>
<td><em>Flammulina velatipes</em></td>
<td>Japan</td>
</tr>
<tr>
<td>Paddy straw mushroom</td>
<td><em>Volvariella volvacea</em></td>
<td>China, Japan</td>
</tr>
<tr>
<td>Ear fungus</td>
<td><em>Auricularia auricula</em></td>
<td>China, Japan</td>
</tr>
<tr>
<td>Reishi mushroom</td>
<td><em>Ganoderma lucidum</em></td>
<td>Japan</td>
</tr>
<tr>
<td>Nameko mushroom</td>
<td><em>Pholiota nameko</em></td>
<td>China, Japan</td>
</tr>
<tr>
<td>White jelly fungi</td>
<td><em>Tremella fuciformis</em></td>
<td>Taiwan</td>
</tr>
<tr>
<td>Truffle</td>
<td><em>Tuber aestivum</em></td>
<td>Europe, New Zealand, Australia</td>
</tr>
</tbody>
</table>
**Mushroom breeding strategies**

Mushrooms have gained the reputation of being difficult organisms to work with and it was widely acknowledged that the mushroom, particularly *Agaricus bisporus* is not easy to manipulate through breeding. During early attempts at genetic improvement in the cultivated mushroom *A. bisporus*, there was not much understanding of the natural breeding system. The mushroom is now known to be a “secondarily homothallic” species with a single multiallelic mating type factor (Elliott and Langton, 1981). This understanding can evaluate the breeding methods previously used and to suggest alternatives. Strain selection based on single spores, multisporous or tissue culture may give improvement in the short term but is not as effective as methods with controlled crossing. Mixing fertile strains may produce hybrids but they are sometimes difficult to identify. It is better to use non-fertile isolates or any of the hybrids; recognition of hybrids can be done using markers that can be expressed only in hybrid cultures and the incorporation of genetic resistant trait is especially useful for this.

**Goals of mushroom breeding**

Increasing the yield and quality of crops as well as resistance to diseases are the primary goals for mushroom breeders and mushroom research. Other goals include reducing production costs and the efficient use of compost for growth. Methods of mass selection based on natural chance mutation and programmed mutation by ionizing radiations such as γ-rays, X-rays and chemicals as well as cross breeding and transgenic breeding are some of the methods carried out for this purpose. However, cross and transgenic breeding are more effective and have shown greater promise and progress in the last few decades (Fan et al., 2006). Areas of research for mushroom breeding relate directly to commercial benefits such as problems associated with cultivation, distribution and storage, senescence-induced browning and disease resistance.

Another aim of breeding in mushrooms is to incorporate various improved crop growing qualities such as shorter growth cycle and prevention of spore formation. Traditionally mushrooms produce billions of spores floating in the air which cause health problems such as lung allergy and fever attacks. Spores also lead to the blocking of climate installations and result in higher energy costs. A new sporeless oyster mushroom has been developed by Plant Research International, Wageningen, Netherlands by using molecular marker technology. After crossing various oyster mushroom cultivars, this analytical technique can be used to rapidly identify progeny with the highest chance of sporelessness (Okuda et al., 2003). Other research areas for crop improvement in mushrooms are utilization of grain-based substrates substituted for traditional manure-based compost which will address issues related to outdoor composting and compost disposal and potentially offer sterilized substrate for biopharmaceutical manufacturing (Bechara et al., 2006). To increase yield, various strategies have been carried out. Many older strains which grow profusely and produce a large number of pins but never mature are hybridized with strains with less growth. Production of hybrids with increased thickness and density of mushroom cap as well as prolific nature of the hybrid strains has increased yield potential. Two methods successfully employed for breeding in the lab are protoplast culture and spore germination (Horgan et al., 1991, Kerrigan et al., 1992). Improvement of strains for commercially grown fungi was boosted 30 years ago with the development of methods for isolation of protoplasts (Chang et al., 1993).

This was performed using microbial enzymes that could digest the fungal cell walls and also provide an osmotic support for the naked protoplast. Methods to fuse protoplast from different sources followed later. Djajangera and Masduki, (2010) carried out protoplast fusion between white and brown oyster mushrooms to obtain high productivity and long storage life. The regeneration of protoplasts is a time consuming and difficult process but has been performed successfully in species of *Pleurotus* and *Volvariella* (Reyes et al., 1998).

**Breeding for disease resistance**

Disease outbreaks can severely reduce the yield and productivity of commercial mushroom production. Many mushroom pathogens are resistant to benzimidazole fungicides or tolerant to neomycin. New pathogens such as *Trichoderma aggressivum*, *Cladosohryum mycophillum* and the mushroom virus X have emerged at regular intervals over the years. Approved pesticides have been significantly reduced in Europe due to consumer and environmental concerns. Moreover, it is difficult to control many fungal diseases since mushroom itself is a fungus. This combination of circumstances makes controlling disease outbreaks in mushrooms more challenging. Disease control primarily depends on effective hygiene measures and sanitation. Knowledge of disease cycle and epidemiology of specific pathogens can provide growers with insight into how they are transmitted and spread. The control of growth environment by regulating the temperature and relative humidity has
resulted in containing the spread of some air-borne fungal pathogens without the application of genetic resistance or fungicides. But the development of fungicide resistant strains and restriction on the use of pesticides has increased requirement for resistant cultivars (Fletcher, 1992). Companies trying to develop disease resistant cultivars or pesticides cannot afford to undertake expensive research programs for these crops. There are thus, few cultivars that are bred specifically for disease resistance and available to the growers. *Agaricus bitorquis* and *A.bisporus* are valuable horticultural crops cultivated throughout the world with numerous strains and hybrids. There is not much work reported on comparing spawn strains and disease incidence and strains marketed that are disease resistant. Van Zaayen and Van der Pol-linton, (1977) studied different strains of *A. bisporus* and found five strains that were less susceptible to false truffle disease (*Diehliomyces microsporum*). Peng, (1986) also noticed differences in disease susceptibility and found strains of *A. bitorquis* that were naturally more resistant to *Verticillium fumigica*. He also found strain variation in response to bacterial blotch (*Pseudomonas tolasii*).

The occurrence of viral diseases in *A.bisporus* varies with strains but it may also be due to incompatibility between strains that prevents anastomosis which is a mode of transmission for the disease inoculum. Selecting and breeding of the naturally resistant strains was carried out. Challen and Elliott, (1987) bred novel strains of *A.bisporus* which were resistant to four fungicides. Research on disease control and diagnosis by PCR tests of major disease-causing organisms of mushrooms such as *Verticillium, Trichoderma* and La France disease virus has been performed in many labs (Chen et al., 1999). The existence of a viral complex associated with La France disease and characterization of three RNA viruses in *A.bisporus* have been identified by molecular methods. The genetic engineering of mushrooms with *Bacillus subtilis* bacteria that can control the growth of disease-causing fungi such as *Trichoderma* is an area of promise for future mushroom research.

**Hybrid breeding**

The hybrid mushroom strains introduced in the 1980s were well received and popular and have limited the choice of production characteristics and range of tolerance to environmental and cultural stresses. Cross breeding has been carried out since 1983 in mushrooms with the production of hybrids in *Lentinula* (Zhang and Molina, 1995), *Pleurotus* and *Agaricus* (Fritsche, 1983). Hybrid strains have not only given mushrooms that show resistance to diseases and pests but also reduced the dependence and risks of environmental and cultural stresses. Hybrids obtained by pairing monosporic cultures are cultivated to evaluate the production characteristics accompanied by RAPD and RFLP analysis. Mushroom breeding requires a large investment of capital and patience from both the breeder and grower. A number of specific industry standards have been adopted to grow strains available to the public for the last 30 years. For a new strain to be successful, some modifications in growing parameters are required for optimal growth. Traditionally growers had to adapt growing systems to accommodate cultural needs such as modifying flushing regimes, watering patterns and harvesting practices to optimize strain performance. Modifying cultural practices such as frequency and timing of irrigations are required for successful future strain development.

**Marker Assisted Selection breeding (MAS) in mushrooms**

Breeders now use DNA molecular markers to identify and select specific genes to locate superior traits. Repetitive DNA sequences are used to generate markers by PCR based techniques (Khush et al., 1991) as well as RAPD methods (Yan and Jing, 2005; Milad et al., 2011) Computer softwares are used to take the experimental data and generate genetic maps (Fouloungne-Oriol et al., 2011). The use of genetic markers is easier for monogenic traits that segregate in distinct phenotypes in mushrooms. Previously white and off-white mushrooms

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**Table 2. Some chemical compounds extracted from mushrooms**

<table>
<thead>
<tr>
<th>Chemical compounds</th>
<th>Obtained from</th>
<th>Properties</th>
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<tbody>
<tr>
<td>Peptido glycans</td>
<td><em>Lentinus, Schizophyllum, Grifola, Sclerotinia</em></td>
<td>Anti-tumour</td>
</tr>
<tr>
<td>Ergosterol</td>
<td><em>Agaricus blazei</em></td>
<td>Anti-tumour</td>
</tr>
<tr>
<td>Lipid fraction</td>
<td><em>Grifola</em></td>
<td>Anti-oxidant</td>
</tr>
<tr>
<td>Steroids, hydroquinones</td>
<td><em>Ganoderma pfefferi</em></td>
<td>Anti-bacterial</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td><em>Lentinula edodes</em></td>
<td>Anti-protozoal</td>
</tr>
<tr>
<td>Schizophyllan</td>
<td><em>Schizophyllum commune</em></td>
<td>Anti-tumour</td>
</tr>
<tr>
<td>Epicorazin</td>
<td><em>Podaxis pistillaris</em></td>
<td>Anti-microbial</td>
</tr>
<tr>
<td>Ganoderic acid</td>
<td><em>Ganoderma lucidum</em></td>
<td>Anti-viral (HIV)</td>
</tr>
<tr>
<td>Ganoic acid, lucadiol,</td>
<td><em>Ganoderma sps.</em></td>
<td>Anti-viral (influenza)</td>
</tr>
<tr>
<td>Lignins</td>
<td><em>Inonotus obliquus</em></td>
<td>Anti-viral (HIV)</td>
</tr>
<tr>
<td>Polysaccardies</td>
<td><em>Trametes versicolor</em></td>
<td>Anti-viral (HIV)</td>
</tr>
<tr>
<td>Velutin</td>
<td><em>Flammulina velutipes</em></td>
<td>Anti-viral (HIV)</td>
</tr>
<tr>
<td>Illudins</td>
<td><em>Omphalotus olearis</em></td>
<td>Cytotoxic</td>
</tr>
<tr>
<td>Triterpenes</td>
<td><em>Ganoderma concinnum</em></td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Polysaccardises</td>
<td><em>Phellinus linteus</em></td>
<td>Antiangiogenic</td>
</tr>
<tr>
<td>Hispolon, hispidin</td>
<td><em>Indocalamus hispidin</em></td>
<td>Anti-allergic</td>
</tr>
<tr>
<td>Ergosterol peroxide</td>
<td><em>Tricholoma populrn</em></td>
<td>Anti-allergic</td>
</tr>
</tbody>
</table>
strains dominated the industry and each strain had certain favourable and unfavourable traits associated with them. The off white strains were better for mechanical harvesting but not conducive to canning due to discoloration whereas the white strains were less prolific but did not discolor on slicing and canning. Fritsche (1986) combined both strains in a breeding program to produce a hybrid with best qualities of each strain. By the introduction of DNA molecular markers, mushroom spawn producers can identify and fingerprint their strains providing greater patent protection and provide resources to expand breeding programs (Zhang et al., 2010a). Today MAS allows for a fast, easy and cheap method for the screening of cultures and selection in mushroom breeding (Kerrigan, 2000). The markers are associated with agronomic traits such as cap shape, color and quality (Miyazaki et al., 2010). MAS can identify the homokaryons with desirable traits without waiting for the fruiting stage, making it easier and faster than conventional breeding. The use of genetic markers for monogenic traits that segregate in distinct phenotypic characters such as cap colour have been used to introduce brown colour of a wild variety into commercial hybrids by introgression breeding. Also, the introduction of spineless trait to commercial strains of Pleurotus was done by this method (Okuda et al., 2009). Complex traits such as yield, disease resistance and quality characteristics are usually inherited quantitatively. These traits are found to be associated with quantitative trait loci (QTL). Many QTL loci that are responsible for causing diseases such as bacterial blotch and dry bubble have been located. Intercrossing and backcrossing has given rise to hybrids that are less sensitive to these diseases. A large number of natural genetic variability already exists in wild populations of A. bisporus and it is not always necessary to modify or genetically engineer strains (Loftus et al., 2000). Thus, utilizing traditional breeding methods to explore traits from wild isolates can expand the genetic base of the cultivated mushrooms. But it is not clear which traits exist in the wild germplasm collection and if they can be successfully bred into commercial strains.

Transgenic breeding

At present there are no transgenic mushroom strains available commercially but several research groups are working towards that direction with good progress. The use of recombinant DNA technique for creating transgenic mushrooms has created numerous possibilities and opportunities. Importing genes from unrelated sources is now possible and it is not restricted to searching for desirable genes only within the species. Transformation techniques used with other filamentous fungi are being adapted for the mushroom (Van de Rhee et al., 1996a). Various techniques such as polyethylene glycol (Li et al., 2006), electroporation and particle bombardment have been used to incorporate DNA into protoplasts, mycelium or basidiospores.

An efficient homologous site-directed integration of the transformation plasmid was done by isolating the tyrosinase genes responsible for mushroom browning from Agaricus bisporus and introducing it in antisense orientation (Van de Rhee et al., 1996b). However, the multinuclear nature of fertile Agaricus mycelia presented a problem for stable transgenic mushrooms. Another gene isolated and identified in mushrooms was the mannitol-dehydrogenase (MIDH) gene and its 3-dimensional structure has now become available (Sassoon et al., 2001). Isolation of this gene can allow the production of mushrooms with altered mannitol profiles and ultimately yield strains with higher dry matter content or better pathogen resistance (Stoop and Mooibroek, 1998). The use of direct gene delivery techniques such as particle bombardment has also been carried out as an alternative method for genetic transformation in mushrooms (Li and Horgan, 1993). This process involves the bombardment of intact tissues with tungsten or gold particles coated with donor DNA and penetrating the recipient tissue. It has the advantage of being less laborious and often the problematic production and regeneration of protoplasts can be avoided. In many laboratories, attempts have been undertaken to introduce hygromycin-B resistance and other selectable markers by particle bombardment. However, this technique has not yet resulted in the selection of stable transformants or an applicable system.

Agrobacterium-based transformation

While many other transformation techniques are not very reliable or stable, the use of the soil bacterium, Agrobacterium tumefaciens for transformation reportedly yields stable transformants. The Agrobacterium system allows transformation of both homokaryons and heterokaryons and both karyotypes of a heteroharyon can be transferred simultaneously (Mikosch et al., 2001). The use of A. tumefaciens for efficient transformation and induction of its virulence gene with the plant hormone acetosyringone was first carried out in Agaricus bisporus by De Groot et al. (1998). But the limitations of this method were that it was not reproducible, showed false positives, low level of integration and DNA modification after integration. Also important is the necessity to include redundant DNA in the transformation vector that have no function in Agaricus but are needed for gene transfer only. A successful Agrobacterium-mediated transformation was done by infecting the fruiting gill tissue with Agrobacterium strains carrying the gene construct of interest and use of a vector with homologous promoter (Chen et al., 2000). In most cases, the multinuclear nature of mushroom mycelia has restricted the advances of genetic breeding to yield strikingly improved features (Stoop and Mooibroek, 1999). Many transgenic manipulations with mushrooms will require the transfer of the gene to both parental lines so that the offsprings mimic the natural inheritance process by carrying duplicate copies of the gene. In others, introduction of a single copy of the gene is sufficient and the resulting transgenic line may require further selection before introduction as commercial strains. Areas for transgenic breeding possibilities include importing cry genes from Bacillus thuringiensis for insect resistance and synthetase resistance from Agrobacterium for glyphosate herbicide resistance.

Sequencing the mushroom genome

With the onset of the Genomics era, the nucleotide sequences of entire genomes have been determined (Yu et al., 2002; Buell et al. 2003). With regard to mushrooms, a proposal honoured by the US Department of Energy and carried out by “Joint Genome Institute” of America is underway for sequencing the genomes of the button mushroom, Agaricus bisporus (Kerrigan, 2009) and also the oyster mushroom, Pleurotus ostreatus (Pisabarro et al., 2006). The proposal draws up a document that
substantiates the importance of sequencing the mushroom genome since mushrooms are now regarded as being very important for the environment. This is in view of the fact that mushrooms help in degradation of plant material into less harmful substances, are also used to remove heavy metals from waste flows and also play a role in production of biofuels (Thwaites et al., 2007). Sequencing work in *Schizophyllum commune*, a wood-degrading fungus was already completed this year (Ohm et al., 2010) by an international consortium of mushroom researchers and will now be available in public domain. After sequencing, investigations will continue into how sets of functionally related genes are organized and clustered into chromosomes. Also how these genes are distributed in related and non-related organisms will be observed. It will open up possibilities as to when and where a particular gene will become active and why. Applying functional genomics to look at gene expression, promoter, transcriptional and other regulator elements by using tools of gene silencing are other areas of research. For breeders using DNA markers, a more detailed and directed breeding of new mushroom strains can now be done. It opens the possibility of identifying regions of DNA associated to more complicated traits such as disease resistance and cellulose metabolism. Breeding advances made in other crops after mapping their genomes can also be realized in case of mushrooms.

**Future scope**

The use of genetic engineering in mushroom industry will be determined by economic factors related to necessity and resources. Due to funding constraints, mushrooms are very much lagging behind other crops in terms of advancement in molecular biotechnology. Public acceptance of genetically modified foods and greater consumption of mushrooms can increase research efforts (Snow and Palma, 1997). Traits controlled by single genes such as viral and insect resistance and resistance to fungal and bacterial pathogens and pesticides can be targeted first since they are simpler to tackle.

With mapping of the mushroom genome and understanding of the functional genomics in mushrooms, complex traits such as yield, size, colour, shelf-life, and physical stress which are controlled by more than one gene can be undertaken in the future. Mushrooms can also be utilized as bioreactors in industry for the synthesis of proteins and pharmaceutical compounds (Tang et al., 2007). A higher biomass of mushrooms can be produced on low-cost waste materials in a secure containment facility with the option of automation and mechanical harvesting (Lee et al., 2002). The proteins manufactured in mushrooms will also have higher specific biological activities in humans than those produced from plants (Lum and Min, 2011). The production of new mushroom cultivars with novel and improved traits will provide the industry with options for solving food problems (Zhang et al., 2010b) and increase the production efficiency. Improvement of tools available to the breeder, decoding mushroom genome and commercial pressure facing the industry can propel efforts for new strain development in the future.

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**References**


