Breeding Mushrooms: State of the Art

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Abstract: Compared to animal and plant breeding, mushroom breeding is a late starter. This is not surprising since the worldwide production of mushrooms is small compared to many plant crops. The production of mushrooms, however, has increased ten-fold worldwide in the last forty years and is increasing now faster than ever before. Mushrooms are attractive organisms for breeding compared to plants. Especially the unique life cycle and short generation time of these fungi allow efficient breeding. Techniques developed in plant breeding are now also adopted in mushroom breeding. These include the use of molecular markers for single- and multi-gene encoded traits. This paper will describe the state of the art in mushroom breeding by discussing the main issues for mushroom breeding and giving examples of breeding in *Agaricus bisporus* and *Pleurotus ostreatus.*

Key words: Breeding strategy, QTL quantitative trait, *Agaricus bisporus, Pleurotus ostreatus*, disease resistance.

1 Introduction

Unlike plant and animal breeding, mushroom breeding is a relatively new applied science. This is not surprising since only recently have mushrooms been produced commercially on a large scale. The annual worldwide production has increased from ca. 300,000 metric tons in 1964 to three million metric tons in 2003 (http://faostat.fao.org). Despite this ten-fold increase in the last 40 years the production is still small compared to many plant crops. The profit from breeding is hence small due to a limited market and hampers the investment of large sums of money in breeding programs. As a consequence, scientific research on mushroom breeding lags behind that of plant and animal breeding. However, the size of their genome, the handling as a micro-organism in a laboratory and the relative short life cycle make edible fungi an attractive organism for breeding. For most fungal species producing edible mushrooms, the important prerequisites for breeding are fulfilled, i.e. their life cycle is known and genetically variable source material is available. The use of markers to select desirable offspring (marker assisted selection, i.e. MAS) is now in common use in mushroom breeding and has led recently to new strains for the button mushroom, *Agaricus bisporus*, and the oyster mushroom, *Pleurotus ostreatus*. MAS has, however, up to now only been used for selection of traits determined by a single locus or gene. No new strains are known that were produced using MAS for selection of quantitatively inherited traits. These types of traits (polygenic) are usually determined by more than one gene and their phenotype is affected by environmental factors. In plant and animal breeding it appears that many economically important traits are determined by QTL (Quantitative Trait Loci), such as yield, quality and resistance to diseases.

Recently a breeding program was started for *A. bisporus* aiming to introduce quantitatively inherited traits from wild collected lines into commercial lines. This program is a co-operation between Applied Plant Research, Horst, The Netherlands and Sylvan Research, Kittanning, USA. The program is funded by Bromyc b.v., a subsidiary company of CNC Compost, The Netherlands, and by Sylvan Inc, USA. To our knowledge, this is the first breeding program for edible mushrooms in which QTL techniques are used.

This paper will describe in general terms a breeding strategy that is common in plant breeding and which also appears to be useful in mushroom breeding. It will describe the state of the art in mushroom breeding by giving examples for the use of molecular markers in introducing single gene and multiple gene encoded traits into *A. bisporus* and *P. ostreatus.*

The purpose of breeding is to combine desired traits present in separate individuals using controlled crosses and selection of offspring. For this the life cycle of the organism must be known and the relevant traits must have a genetic basis. In addition one should have an efficient breeding strategy since financial resources are usually limited. A brief outline of the life cycles of Homobasidiomycetes, to which most mushroom producing species belong, will be given.

2 The Life Cycle of Mushrooms

The typical life cycle of Homobasidiomycetes is characterized by the fact that most of its life cycle is haploid. Fusion of nuclei only takes place in basidial cells just before spores are produced. Each diploid nucleus produces four haploid nuclei after meiosis and these are distributed to the four spores formed by each basidial cell. The spores germinate into haploid mycelium that cannot produce fruiting bodies. These infertile mycelia are designated as homokaryons (monokaryons). Homokaryons with opposite mating type can anastomose and nuclear exchange leads to the formation of heterokaryotic (dikaryotic) mycelium. The presence of both mating types within one mycelial cell triggers a developmental process leading to the formation of fruiting bodies provided environmental conditions are favorable. This non-self compatibility or heterothallism is controlled by one (unifactorial) or two unlinked loci (bifactorial). This system restricts inbreeding to 50% or 25% in unifactorial and bifactorial systems, respectively.[1] The outbreeding potentials of basidiomycetes are high because they possess numerous distinct mating types.[2]
The majority of Homobasidiomycetes have either of the two types of heterothallic life cycles. Opposite of this is the self-fertile homothallic life cycle. Here, also two forms can be distinguished. In true or primary homothallic species life the cycle is completed in the absence of mating. Spores are haploid and form sterile homokaryons that can produce fruiting bodies. In secondary homothallic species, most basidia produce two spores. Meiosis takes place after fusion of nuclei in the basidial cell and the non-sister nuclei are usually paired in one nucleus. This leads to mycelia with opposing mating types and thus to fertile heterokaryons. The cultivated button mushroom *A. bisporus* is an example of a species representing both heterothallic and secondarily homothallic life cycle. All commercially produced strains and most wild strains have a predominantly secondarily homothallic life cycle. Only a small portion of the basidia produces 3 or 4 spores that form infertile homokaryotic mycelium. Recently, Kerrigan and Callac described a novel tetrasporic variety of *A. bisporus* from the Sonoran desert of California.[3] Most of the basidia of this variety produce four spores that are haploid and mating between compatible homokaryons leads to fertile heterokaryotic mycelia. Both varieties are completely interfertile and designated as *A. bisporus* var. *bisporus* and *A. bisporus* var. *burnetti,* respectively. It has been estimated that approximately 85-90% of the Homobasidiomycetes have a heterothallic life cycle with bipolar or tetrapolar compatibility system. The remaining 10-15% is homothallic or secondarily homothallic. An important conclusion is that most species used to produce mushrooms have a life cycle that allows breeding.

3 Source Material

A prerequisite for breeding is the availability of genetically diverse source materials. For *A. bisporus* a collection of wild isolates is available.[4] This collection consists of strains that have diverse traits described by Sonnenberg et al.[5] and Kerrigan.[6] Main characteristics that have relevance to breeding are cap color, temperature tolerance, disease resistance and differences in cultivation characteristics such as variation in flushing pattern and number and weight of fruiting bodies. Especially the presence of strains that have low sensitivity to major diseases is important to breeders. An increasing consumer concern for the application of chemicals and government policy to reduce the use of any chemical in agricultural crops underlines the need for research on alternatives such as resistant strains or alternative protective agents from natural sources.
APR also has an extensive wild collection of *P. ostreatus* strains in addition to a collection of commercial

strains that have been or are used since 1960 for commercial production. The wild germplasm of this species has not yet been exploited but a genetic fingerprint of these lines has shown that the genetic diversity is large, contrary to the low genetic variability of commercial strains.[7]

4 Breeding Strategies

Breeding starts with the selection of lines representing the traits of interest. Crosses are then made and offspring selected with a desired combination of traits. In plant and animal breeding, gametes of both parental lines are needed to generate hybrids. Each gamete represents a recombinant haploid genome derived from the diploid parental nucleus. At the beginning of a breeding project, one has no knowledge of the genetic basis of a trait and thus many gamete combinations (crosses) have to be generated to be sure that the genetic basis for the trait is present in the breeding stock. Mushroom breeding can be more straightforward and less laborious with this approach. The reason lies in the typical life cycle that has been pointed out in the previous paragraph. Nuclei of different mating type do not fuse until just before the generation of spores. The presence of both nuclei in heterokaryotic mycelium allows thus for isolation of the constituent homokaryons.[8] If successful, breeding can start with two homokaryons representing the complete genetic make-up of the selected line. However, not in all cases can both parental homokaryons be recovered in this way. An alternative way of combining non-recovered parental nuclei with other homokaryons is by making use of the "Buller phenomenon", a mechanism for nuclear exchange between homo- and heterokaryons previously described by Raper.[9]
A very common strategy for breeding is the introduction of a new trait derived from a wild line (donor strain) into an existing commercial line (recipient strain). If all four constituent homokaryons of both lines have been recovered, a straightforward breeding strategy can be used (Figure 1). Compatible homokaryons of the two selected lines are mated and fruited. Homokaryotic offspring are obtained as single spore isolates (SSIs) and tested for the traits of interest. Since most traits are only displayed by mushrooms, the selected SSIs must be mated to a tester homokaryon and fruited to study the segregation of the trait. A suitable tester strain is one of the parental homokaryons not involved in the relevant cross, and which contributes little or nothing to expression of the trait of interest. The set of offspring should be large enough to observe the full range of the phenotypic expression. Especially quantitative traits need large sets of offspring and repeated testing since variation in expression is not only caused by genetic variation but also by environmental factors. Depending on the dominance or recessiveness, the trait is introduced into one or both parental homokaryons of the recipient strain by repeated backcrossing and selection for the trait. This so-called introgression of traits in parental lines restores most of the original genetic make-up of the recipient parental line and reduces the introduced donor genome to relevant parts that harbor the trait. As a final step, the introgressed parental lines are mated and a new hybrid line is constructed that has acquired a new trait and has retained most of its original characteristics.

4.1 Genetic markers and generation of genetic maps

By studying the segregation of genetic markers and the relevant trait in the offspring, genetic markers can be found that are linked to gene loci that underlie the trait of interest. This allows for the selection of offspring with the desired trait without the need for testing of phenotypes, which can be elaborate, slow, and costly. The first step is the generation of a genetic map. This map can be constructed by studying segregation of markers in offspring derived from a cross between the selected parental lines. Plant breeders use backcrosses or F2 intracrosses between selected lines to generate a mapping population in order to obtain plant material that can be used to study marker segregation in post-meiotic products. In mushroom breeding, however, one can obtain individual post meiotic products directly from each generation. Diluted spore suspensions are plated out and single spore isolates (SSIs) are vegetatively propagated to obtain sufficient DNA for the segregation analysis of genetic markers. For heterothallic species most of the SSIs are haploid and can thus be used for the linkage

analysis. For secondarily homothallic species such as *A. bisporus* var. *bisporus* this is somewhat problematic. Only a low percentage of the SSIs are homokaryotic and there is no clear morphological distinction between homo- and heterokaryons. Homokaryotic SSIs, however, grow generally slower than heterokaryotic SSIs. By a pre-selection for slower growth, usually sufficient homokaryons are obtained.[10, 11] The tetrasporic trait in the variety *A. bisporus* var. *burnetti* has been mapped to chromosome 1 and the trait is dominant in a bisporic/tetrasporic hybrid.[12] This allows the introduction of the trait in all breeding lines of *A. bisporus* and thus facilitates the selection of haploid SSIs.

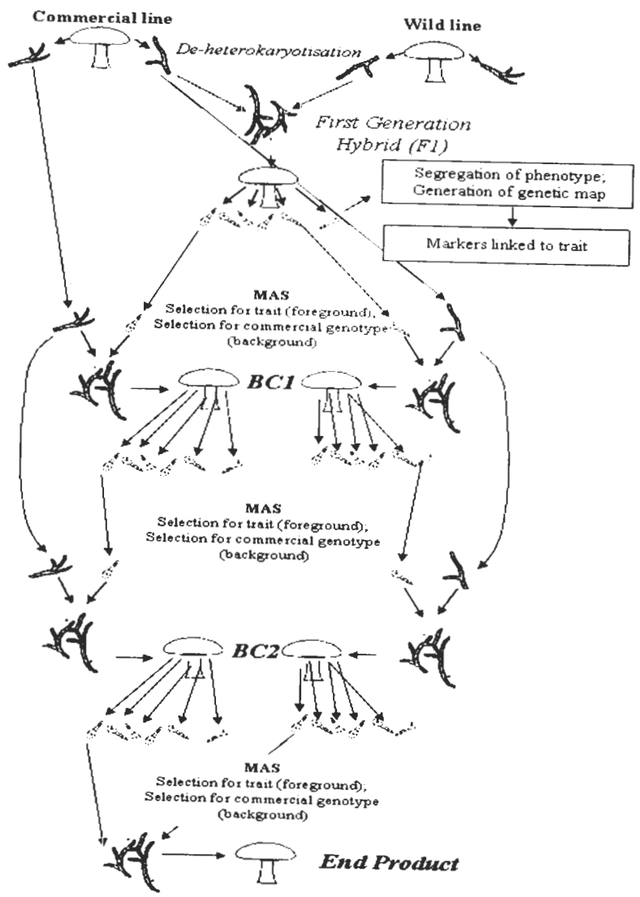

Figure 1. Example of a breeding strategy for introgression of traits of a wild line into a commercial strain

Relevant genetic information present in one of the constituent parental lines of the wild isolate is introduced into both parental lines of the commercial strain via two backcrosses (BC1/BC2) . Genetic markers are used to select for the trait (foreground) and for commercial genome (background).

There has been an enormous increase during the last decades in the efficiency of generating DNA markers. Previously, isoenzymes and restriction fragment length polymorphisms (RFLPs) were commonly used as genetic markers.[13] The advantage of these markers is their codominancy (i.e. both alleles can be visualised) and thus the phase of a marker is always known in the offspring. The use of these types of markers is, however, not very efficient because only one or very few markers can be scored at a time. Most markers used nowadays are

generated by PCR based techniques. Repetitive DNA sequences are in this respect very useful because a number of markers can be generated in each individual PCR reaction.[14] Advanced computer software is available that can use the experimental data to generate publication-ready genetic maps.[15, 16] Segregation analyses that we have done so far in *A. bisporus* and *P. ostreatus* indicate a frequency in the genome between 2 and 5 recombinations per generation. In our experience, an offspring set of 100 SSIs is then sufficient to generate a robust genetic map for these species.

4.2 Linkage of traits and genetic markers

In order to be able to use genetic markers to select desirable offspring, a breeder has to assess with a certain degree of accuracy the association between markers and traits. The association is determined by statistical analyses and thus sample size and repetitions are important. Three parameters are relevant to this accuracy, the size of the segregating population, the density of the genetic map (number of markers per chromosome) and the accuracy with which the trait is assessed. The first two parameters are important for precise mapping of the trait. This will help to reduce the donor genome content in the recipient parental line and thus unwanted traits. The use of genetic markers is straight forward for monogenic traits that segregate in distinct phenotypes such as cap color in mushrooms.[14, 17] The selection for a marker tightly linked to the trait goes along with the selection for the trait. Usefulness of markers for the reduction of donor genome content is amply demonstrated in simulations.[18] When introducing a "one gene trait" in a backcross strategy, marker assisted selection helps to reduce the number of backcrosses by a factor of 2. That number increases enormously with an increase of genes to be introgressed.

Complex (multigenic) traits such as yield, resistance to diseases and quality characteristics are usually inherited quantitatively. They show a nearly continuous variation of a phenotype among segregating offspring. Often the trait depends on more than one gene and the phenotypic effect of each associated gene to the trait is relatively small. In addition, the phenotypic variation in the offspring also depends on environmental factors. It is, therefore, important to assess the trait in several independent experiments under standardized conditions. That allows a more accurate estimation of the heritability of the trait. It is only by studying the joint segregation of genetic markers and phenotypes in offsprings that the detection and localisation of genetic loci that underlie these types of traits becomes possible. These loci are designated quantitative trait loci (QTL). Especially, the development of molecular markers and powerful biometric methods has led to considerable progress in QTL developments. For quantitative traits more than one QTL is normally found. Examining QTL analyses in 176 trial-traits of plants described in several earlier publications, Kearsey & Farquhar[19] found an average of 4 QTL per trait, with a range from 1 to 16. So far only two QTL analyses have been published for mushroom breeding. Larraya et al.[20] have found 1 to 4 QTL for each of several diverse agronomic traits in the oyster mushroom *P. ostreatus*. Moquet et al.[21] found only one QTL for sensitivity to bacterial blotch caused by *Pseudomonas tolaasii.* QTL that make minor contributions to the trait phenotype may not be detectable with statistical confidence unless very large sets of offspring are analyzed.

To estimate the genomic position of a QTL, the segregation of the trait is studied relative to marker pairs rather than single markers. In this so-called Interval Mapping, "bracket" markers are used that give a better estimate for the position and the size of the genomic region of the QTL. Next to this, mapping software has been designed to reduce the residual variation in each QTL caused by other QTL for the relevant trait. With this so-called Multiple QTL Mapping (MQM) better estimates are also obtained for QTL.[22] Nevertheless, genomic regions assigned to QTL are rather large. For plant breeding it has been estimated that on average QTL regions are 30 cM or more.[19] Considering that, on average, chromosomes have a length of 100 cM, this is a large portion of a chromosome. For a more precise mapping of QTL enormous numbers of offspring should be isolated to generate offspring that contain different subsets of each QTL region, and all should be tested phenotypically. That, however, is beyond most breeding program budgets. Improving strains using QTL demands,

therefore, efficient breeding programs.

The ultimate goal of a breeding program is to introduce only the relevant parts of the donor genome while restoring/retaining the genome of the recipient parental line as much as possible. One can thus select for the trait (foreground) and for the recipient parental genome (background). The latter requires selection for background on carrier chromosomes (harboring the trait) and on non-carrier chromosomes. Thus, the question is what strategies are optimal to reach the goal in a breeding program with the highest efficiency, i.e. the lowest costs and/or the fastest progress. A number of publications have appeared on these kinds of strategies in plant breeding based on simulations.[18, 23-25] These papers deal with issues such as the number of backcrosses needed, population sizes in offspring, marker densities and strategies for single and multiple gene/QTL introgression. It is beyond this paper to deal with these complex mathematical issues. Basically, a multi-stage selection of markers[25] is suggested in most papers. A first selection is done for markers linked to the trait or gene (foreground) followed by a selection for background markers i.e. a selection for recipient parental markers for carrier and non-carrier chromosomes (background). The authors also consider a number of variations on this theme.

For mushroom breeding one trait is very important, i.e. the mating type. As mentioned previously compatibility between homokaryons is determined by one locus or two unlinked loci. Only homokaryons of unlike or 'opposite' mating types are compatible. Co-dominant markers, tightly linked to the mating type, are very useful with this respect. For breeding pseudohomothallic strains of *A. bisporus* such markers can be used to discriminate between homo- and heterokaryons. The recombination frequency between the mating type locus and the centromere appears to be very low in *A. bisporus*[26] (Sonnenberg, unpublished results). This leads to the segregation of mating types in the first meiotic division. As mentioned in the paragraph on the life cycle of basidiomycetes, in *A. bisporus* var, *bisporus* two-spored basidia are common and three to four-spored basidia are rare. In the two-spored basidia, non-sister nuclei are paired in the same nucleus and, thus lead to heterokaryotic mycelia containing both mating type genes. Co-dominant polymorphic markers tightly linked to the mating type locus are thus useful to discriminate between homo- and heterokaryotic mycelia. In addition, these markers will also reveal the phase of the mating type and thus provide information on the compatibility of a particular individual in the offspring. This information is important in all breeding programs. In introgression programmes, for example, 50% of the offspring are compatible to the recipient parental line and can be backcrossed in unifactorial systems. In bifactorial systems only 25% of the offspring can be backcrossed. It is, therefore, wise to start a selection with a marker that is linked to the mating type.

5 Examples for Marker Assisted Selection Breeding in Mushrooms

We will present here two examples of breeding programs that used MAS for the introduction of a single trait using an introgression breeding program. Another example will be discussed where QTL have been located and subsequently MAS used to introgress the multilocus trait into a commercial line via introgression breeding in *A. bisporus.*

5.1 The use of MAS for single locus traits

An important marketing character of button mushrooms is cap color. Approximately 80% of these mushrooms produced worldwide are white. There is, however, a slowly increasing interest in brown mushrooms, especially the fully matured mushrooms known as Portabello's. Commercially available brown mushroom strains are either identical or genetically closely related (Sonnenberg, unpublished). Compared to the large white hybrids, they are less productive and have a lower quality, i.e. less firm tissue and a shorter shelf life. Previously genetic analyses have shown that the cap color is mainly determined by one single locus on chromosome 8 and that the brown allele is dominant.[17] The authors have found an isoenzyme marker linked to the trait. Later, we have

generated molecular markers based on repetitive elements that are very tightly linked to the cap color.[14] These markers have been used to introduce the brown cap color of a wild line into a commercial large hybrid via introgression breeding. For this the wild line was de-heterokaryotised via protoplasting and both constituent homokaryons were recovered. One of the wild homokaryons was crossed with one of the constituent homokaryons of Horst U1. The cap color was introgressed into this line by two backcrosses. Before each two backcrosses a multi-stage selection was done. First a marker was used for the mating type. This allows for a selection of all homokaryons and, in addition, those that are compatible to the recipient commercial homokaryon. Subsequently a marker was used that was tightly linked to the color locus on chromosome 8 and a selection was made for the wild allele. Finally, a number of markers were scored for the background. For these, 84 repetitive markers were used that could be scored by 15 PCR reactions as previously described by Sonnenberg.[14] These markers were evenly distributed over thirteen chromosomes of *A. bisporus*. In each round, approximately 50 SSIs were screened. Among the offspring of the second backcross, the selection was directed to the mating type of the commercial parental line. Estimating the number of alleles present in the commercial phase, approximately 80% of the recipient commercial genome could be restored in two backcrosses. This indicates the power of marker selection for foreground and background. The recombinant parental line was subsequently crossed to its original parental counterpart and the resulting brown hybrid was evaluated. Three commercial growers have cultivated the new line and compared it with a commercial brown strain. The new strain had different brown color than the commercial brown strains and tends to scale more. It had, however, a 25% higher yield, a high average weight per fruiting body and an excellent shelf life.

Cultivation of oyster mushroom, *P. ostreatus,* is accompanied by an enormous production of spores. Most people that handle and harvest this crop develop respiratory diseases.[27-29] Due to the enormous spore production, climate systems tend to clog and growers do not recirculate air because of this problem. A sporeless strain would allow recirculation and thus save energy. The development of a sporeless strain would, therefore, have a clear commercial and health value.

To introduce the sporeless trait, a sporeless strain of *P. ostreatus* described by Eger[30] has been used. Since Dutch oyster mushroom growers prefer large fleshy fruit bodies, strain HK35 was chosen as a recipient line for the sporeless trait. Test matings of SSIs have shown that mating types of both lines are fully compatible. Protoplasting was used to recover the homokaryotic constituents of the sporeless strain and the sporulating commercial strain HK35. Both homokaryons were recovered from the sporeless strain but only one homokaryotic type could be recovered from the HK35 strain. Previous analysis[30] has shown that the sporeless trait is recessive. It was, therefore, necessary to introduce the trait via introgression in both constituent homokaryons of HK35. One of the homokaryons of the sporeless variety was crossed with the recovered homokaryon of HK35. A mating of the other sporeless homokaryon with the non-recovered homokaryon of HK35 was made by using the "Buller phenomenon" mentioned earlier. Both hybrids produced spores, indicating that the trait is indeed recessive. SSIs were used to generate a genetic linkage map. In addition, SSIs were mated with the sporeless homokaryon of the opposite breeding line to study the segregation of the sporulation. In this way the trait was mapped to one locus in both homokaryons of the sporeless strain. As with the construction of the brown button mushroom strain described in the previous paragraph, here also multi-stage selection was used to select for mating type, the sporeless trait and the commercial background. Two backcrosses were used and in each approximately 300 SSI offspring were evaluated. The resulting products were tested on a small scale in our test facilities. The two most promising lines were selected and tested on three commercial farms. One of these lines appears to be very similar to the original HK35 in yield, production profile and quality. This line may soon be available as commercial spawn.

Both examples show clearly the power of MAS in mushroom breeding.

5.2 The use of MAS for multi locus traits

In 2002, a breeding program was started for the button mushroom *A. bisporus* with the intention to introduce quantitatively inherited traits from wild collected lines into commercial lines. The focus lies on new lines resistant to two of the most important pathogens in mushroom crops, i.e. *Verticillium fungicola,* the cause of dry bubbles, and *Trichoderma aggressivum* spp., causing severe infection problems in compost resulting in crop loss. Here we will confine ourselves to the progress made in breeding new strains with a lower sensitivity to *V. fungicola* var. *fungicola*

Figure 2. Examples of breeding products obtained after introgression of a single trait into a commercial line using marker assisted selection (MAS)
Left, a sporeless strain of the oyster mushroom (*P. ostreatus*) and right, a brown hybrid of the button mushroom *A. bisporus.*

Kerrigan[31] reported that inheritance of the resistance/low sensitivity trait in offspring of Sylvan strain S600 had the characteristic distribution of a polygenic trait. At the start of the breeding program this was all that was known of the genetic basis of resistance to dry bubble. Dragt et al.[32] showed that in the wild collection of *A. bisporus*, the ARP collection,[4] strains are present with a low sensitivity to *V. fungicola.* Later, we have done a large scale screening on sensitivity of wild lines to *V. fungicola* by infecting crops immediately after casing. A wide range of sensitivity was found varying from a low percentage to 100% of the mushrooms showing symptoms after three flushes (Figure 3). No absolute resistance was found. However, the highest resistance found is such that, when introduced into commercial lines, hygiene should be enough to control the disease without the use of chemicals. Previous experiments have shown that sensitivity of *A. bisporus* strains to *Verticillium* can only be tested on mushrooms and not on mycelium. It also appears that the infection levels vary considerable from crop to crop for unknown reasons. That means that the assessment of the trait is a laborious task.
One of the most resistant strains of the screening program was protoplasted and both constituent homokaryons could be isolated. As a recipient line, one of the large commercial hybrids was chosen for which both parental lines were available. The wild and commercial lines have different mating type alleles and thus all possible pairings between these lines could be made. Each homokaryon of the resistant line was mated with the same homokaryon of the commercial line, fruited and tested for resistance. In this way, the expression of the trait can be tested against a similar genetic background. Both hybrids showed a clearly lower sensitivity to *V. fungicola* but were not as resistant as the original wild line. Also, one of the hybrids was more sensitive than the other. This indicates that the trait has at least some dominance and that both homokaryons of the wild line contribute to the trait albeit to a different extent. With this knowledge a breeding program was set up to map the trait in

both constituent homokaryons of the resistant wild line and to introgress the relevant genetic components into the parental lines of a large commercial hybrid. A scheme was used similar to that illustrated in Figure 1. The segregation of the trait was studied in three independent trials. We will not deal here in detail with the technique of QTL analysis itself and refer to some excellent review papers on plant breeding using QTL.[19, 33] Linkage analyses were done here by using MapQTL.[16] We have found two significant QTL in one homokaryon of the

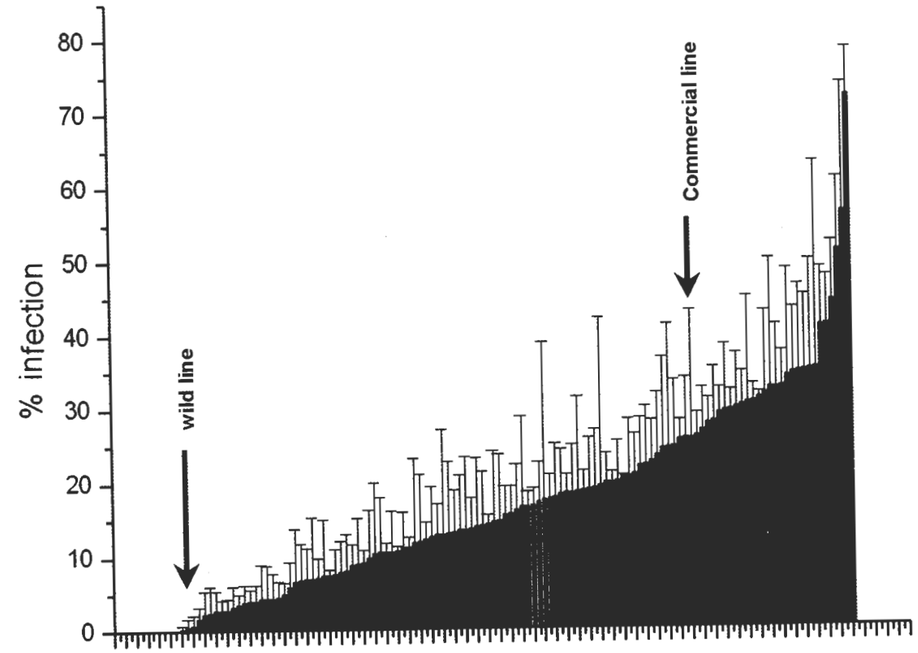

Figure 3. Infection levels in wild and cultivated strains of *A. bisporus* after infection of the casing soil with *Verticillium fungicola*
Infection levels are indicated as percentage of mushrooms showing symptoms after three flushes. The infection levels of the lines used for breeding are indicated by arrows. Standard deviations are indicated for each tested line.

wild line and five QTL in the other wild homokaryon. All markers linked to these QTL were in the wild phase indicating that indeed the genetic information for *Verticillium* resistance is located in the wild line. The confidential interval of each QTL (region on a chromosome were the QTL is located with 95% confidence) varies between 20 to 35 cM. On average, chromosome lengths in *A. bisporus* were 60 to 70 cM (with a range from 30 to 80cM). This indicates that, as in plant breeding, mapping of QTL is rather imprecise. The discovery of seven different QTL, covering large regions of the wild genome, makes a breeding program rather complex. After all, the goal of a breeding program is to introduce the maximal achievable resistance without the loss of desired traits present in the recipient line. The introduction of too much wild genome will certainly lead to a loss of such traits to some extent. An evaluation of crosses between the parallel breeding lines during each generation can help to examine the overall improvement of qualities in each generation and the effect of each QTL on the expression of the trait. In this way an acceptable compromise may be achieved.
Offspring of the first backcross of each breeding line were screened with a multi stage selection as described previously. This resulted in limited numbers of SSI that contained all QTL or were missing one or two QTL. To evaluate the effect of selection for QTL and the variation in numbers of QTL on the trait, a matrix was designed to intercross SSIs of the backcross of the two parallel breeding programs. Each selected SSI offspring of one

backcross was mated to all selected SSI offspring of the other backcross and vice versa. All hybrids were challenged to the pathogen as described. In this way, the performance of each SSI in various hybrids can be evaluated by averaging the column or row infection levels. After challenging the hybrids to the pathogen a significant positive correlation was seen between the number of QTL present in the particulate hybrid and the extent of resistance. This points to an additive effect of the QTL to the trait. From both breeding lines the best SSI, having all QTL and the best performance in the matrix test, was crossed and evaluated in a new trial. Here, the strain was challenged by placing trays in a room with a highly infected crop. As controls, the resistant donor strain and the sensitive commercial recipient strain were enclosed. In this way strains will develop symptoms by secondary infections, a situation that often occurs in crops. It appeared that the new hybrids were less sensitive to secondary infection that the original donor strain (Table 1). To our knowledge this is the first time that selection for QTL in mushroom breeding has been shown to be effective in a breeding program. Large numbers of offspring are now generated to isolated SSIs with the proper recombinations. Similar breeding programmes are now carried out for resistance to *T. aggressivum* and for traits related to cultivation characteristics.

Table 1. Percentage of infected mushrooms after infection with *Verticillium fungicola*

Strain	% Infected mushrooms
Wild	6.5
Commercial	16.0
Hybrid *	0.7

*Hybrid is selected from offspring of cross between the wild and commercial strain using QTL markers for *Verticillium* resistance.

6 Conclusion

We hope that this paper has shown that marker assisted breeding, a common practice in plant and animal breeding, has also excellent potential in mushroom breeding. Even for complex traits, such as resistance to diseases, markers can be found that are of inestimable value for mushroom breeding.

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