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Regular Articles

Quantitative trait locus mapping for bruising sensitivity and cap color of *Agaricus bisporus* (button mushrooms)

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ABSTRACT

White button mushrooms discolor after mechanical damage of the cap skin. This hampers the development of a mechanical harvest system for the fresh market. To unravel the genetic basis for bruising sensitivity, two haploid populations (single spore cultures) were generated derived from crosses between parental lines differing in discoloration after mechanical damage (bruising sensitivity). The haploids were crossed with different homokaryotic tester lines to generate mushrooms and allow assessment of the bruising sensitivity in different genetic backgrounds. Bruising sensitivity appears to be a polygenic highly heritable trait (H^2: 0.88–0.96) and a significant interaction between genotypes and tester lines and genotypes and flushes was found. Using SNP markers evenly spread over all chromosomes, a very low recombination was found between markers allowing only assignment of QTL for bruising sensitivity to chromosomes and not to sub-regions of chromosomes. The cap color of the two parental lines of population 1 is white and brown respectively. A major QTL for bruising sensitivity was assigned to chromosome 8 in population 1 that also harbors the main determinant for cap color (brown versus white). Splitting offspring in white and non-white mushrooms made minor QTL for bruising sensitivity on other chromosomes (e.g. 3 and 10) more prominent. The one on chromosome 10 explained 31% phenotypic variation of bruising sensitivity in flush 2 in the subpopulations of population 1. The two parental lines of population 2 are both white. Major QTL of bruising sensitivity were detected on chromosome 1 and 2, contributing totally more than 44% variation of the bruising sensitivity in flush 1 and 54% variation of that in flush 2. A considerable consistency was found in QTL for bruising sensitivity in the different populations studied across tester lines and flushes indicating that this study will provide a base for breeding cultivars that are less sensitive for bruising allowing the use of mechanical harvest and automatic postharvest handling for produce for the fresh market. The low recombination between homologous chromosomes, however, underlines the need to introduce a normal recombination pattern found in a subspecies of the button mushroom.

1. Introduction

The homobasidiomycete *Agaricus bisporus* (button mushroom) is one of the most widely cultivated and consumed mushroom over the world, especially in western countries (Chang and Wasser, 2012). Mechanical harvesting is more and more used by large mushroom producers worldwide. For instance, nearly 60% of the production is harvested mechanically in the Netherlands, and the rest is handpicked (Straatsma et al., 2007). In practice, only 30–35 kg/h can be picked by hand whereas the capacity of the mechanical harvesting system is up to 400 times higher than that of handpicking. Mechanical harvest is done in a later developmental stage of mushrooms compared to handpicked mushrooms. Mushrooms harvested mechanically have therefore a lower quality and price. During mechanical harvest and handling most mushrooms are bruised and consequently discolor (browning) and are, therefore, mainly used for canning and processing. Up to now, mushrooms of the fresh market are mainly handpicked and a

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mechanical harvesting system for the fresh market, such as a robot picking device, would reduce labor costs dramatically. A fully automated mechanical harvesting system for the fresh market might be feasible, but requires also the availability of strains that are less sensitive to mechanical bruising.

Mushroom browning is a complex process, which can be induced and influenced by a number of environmental factors such as postharvest storage, mechanical damage and the attack of pathogens (Jolivet et al., 1998; Soler-Rivas et al., 1997; Stoop, 1999). The final product that determines the brown discoloration of mushrooms is melanin. Many enzymes are involved in the synthesis of melanin. The polyphenol oxidase (PPO or tyrosinase), involved in the last steps of the melanin synthesis have been studied in detail in the past (Burton et al., 1993; Gerritsen et al., 1994; Mauracher et al., 2014a, 2014b, 2014c; Van Leeuwen and Wichers, 1999). Based on sequence similarities to known pathways, a total of 42 genes involved in the melanin synthesis pathway have been identified in the genome of *A. bisporus*. These are distributed on almost all chromosomes apart from chromosome 6, 11 and 13 (Weijn et al., 2013a). Expression levels of 30 genes were tested, but no clear differential expression was seen between bruising-tolerant and bruising-sensitive strain (Weijn, 2013). Although PPO related genes are considered as potentially the most important candidates involved in mushroom browning it is unknown so far which key genes control the bruise-induced discoloration and which genes are responsible for the observed variation in bruising sensitivity among mushroom strains. To unravel the genetic basis for discoloration after mechanical damage, we therefore used a different approach by studying segregation of bruising sensitivity in offspring of varieties that differ in sensitivity.

A. bisporus is a secondarily homothallic basidiomycetes. Two infertile homokaryons of *A. bisporus* with different alleles at the mating type locus are able to fuse and generate a fertile heterokaryon (Raper and Raper, 1972; Xu et al., 1993). Based on the number of spores formed on the basidia, *A. bisporus* can be differentiated into bisporic and tetrasporic varieties. The two varieties are designated as *A. bisporus* var. *bisporus* and *A. bisporus* var. *burnetti*, respectively (Callac et al., 1993). All commercially cultivated lines and most wild collected strains are *A. bisporus* var. *bisporus* types due to the inferior quality of the *burnettii* type.

The haploid genome size of *A. bisporus* is 30.4 Mb, and it contains 13 chromosomes and has been sequenced completely (Morin et al., 2012). Genetic linkage maps have been reported previously in both bisporic populations of *A. bisporus* var. *bisporus* and intervarietal populations, i.e. *bisporus* × *burnetti* (Foulongne-Oriol et al., 2010; Kerrigan et al., 1993). It has been shown that meiotic recombination is suppressed and very low in *A. bisporus* var. *bisporus*, i.e. less than 0.2 crossover per linkage group. In contrast, the recombination frequency seems to be normal in *A. bisporus* var. *burnetti* since offspring of the intervariety *bisporus* × *burnettii* show a normal recombination frequency of almost one crossover per linkage group. The suppressed recombination frequency results in co-segregating markers and subsequently short and unsaturated genetic linkage maps. The resulting linkage drag hampers introgression breeding for *A. bisporus* var. *bisporus*. This is one of the reasons that new varieties have not been generated since the first hybrids were produced in 1980, i.e. Horst U1 and U3 (Fritsche, 1981). Improvement of the first varieties have been restricted to small modifications by screening fertile single spore cultures (Sonnenberg et al., 1999). Due to a low recombination rate and pairing of non-sister nuclei (Kerrigan et al., 1993), these fertile single spore cultures have a genetic make-up very similar to the original variety and thus also similar phenotypes (Sonnenberg et al., 2011).

Breeding advanced cultivars with superior traits are key to the sustainable development of mushroom industry. Many important economical and agronomic traits in mushroom cultures are quantitative traits controlled by multiple genes or quantitative loci (QTLs). So far, QTL studies in the button mushrooms are scarce. Some trait-related genes or genome loci have been identified in *A. bisporus*, e.g., a major locus for cap color (Callac et al., 1998), QTLs for the disease resistance, e.g. *Pseudomonas tolaasii* and *Lecanicillium fungicola* (Foulongne-Oriol et al., 2011; Moquet et al., 1999; Sonnenberg et al., 2005) and QTLs for yield-related traits (Foulongne-Oriol et al., 2012).

In a previous study, a large scale screening on bruising sensitivity (BS) of a collection of wild strains and commercial cultivars has been done. A wide variation of BS was found, and some wild strains were much less sensitive than commercial strains (Weijn et al., 2012). Strains representing the extremes of BS were selected and protoplasted to recover the constituent nuclei as homokaryotic parental lines. To estimate the breeding value of these homokaryons, they were crossed in all possible combinations (diallel matrix) and the general combining ability (GCA) was determined by assessing BS of mushrooms of each heterokaryon (Gao et al., 2013). The study also helped to select lines for the generation of segregating population for detecting QTL for BS.

The aim of this study was to identify and locate QTL involved in the genetic mechanism of bruising sensitivity in *A. bisporus*. Four representative homokaryotic lines (derived from two white varieties and a brown variety) from the previous study were selected as parental lines and crossed to generate two heterokaryons. Homokaryotic offspring of these two heterokaryons were used for QTL analysis on bruising sensitivity. Like bruising sensitivity, cap color is also determined by the presence or absence of melanin in the outer layer of the mushroom cap. Although discoloration was measured relative to the non-bruised area and thus corrected for cap color variation, the latter might interfere in the correct measurement of discoloration. We assessed, therefore, also QTL for cap color. This study will provide a genetic basis for breeding button mushroom strains that are less sensitive to mechanical bruising.

2. Materials and method

2.1. A. bisporus strains and segregating populations

All strains used in this study were obtained from the fungal collection of Plant Breeding Wageningen UR. Three original heterokaryotic lines were involved, i.e., WB2 a brown wild line resistant to bruising, Horst U1 a white commercial line resistant to bruising, and a white wild line WW7 sensitive to bruising (Weijn et al., 2012). One of the constituent nuclei (homokaryon) of line WB2 (Mes09143) and both constituent nuclei of line WW7 (Z6 and Z8) were recovered through protoplasting the vegetative mycelia. The constituent nuclei of Horst U1 (H97 and H39) were available from the original breeding program.

Four homokaryons recovered from the original heterokaryotic lines were crossed to generate two heterokaryons, which were subsequently used to generate two segregating populations (Fig. 1). Individuals of populations were randomly selected from single spore isolates (SSIs). Homokaryotic SSIs (homokaryons) were identified by PCR method (Gao et al., 2013). Population 1 represents 200 homokaryons of a heterokaryon constructed by crossing H97 and Mes09143. Population 2 represents the 200 homokaryons of heterokaryon formed by H39 and Z8. The two homokaryotic populations were used to generate two linkage maps. For phenotyping, homokaryons must be mated first with a compatible homokaryon, resulting in heterokaryons that can produce fruiting bodies. The parental homokaryons H39, H97 and Z6 were used as tester lines. Population 1 (offspring of H97 × Mes09143) was crossed with H39 to generate heterokaryon Set 1, and the same population was crossed with tester line Z6 to generate

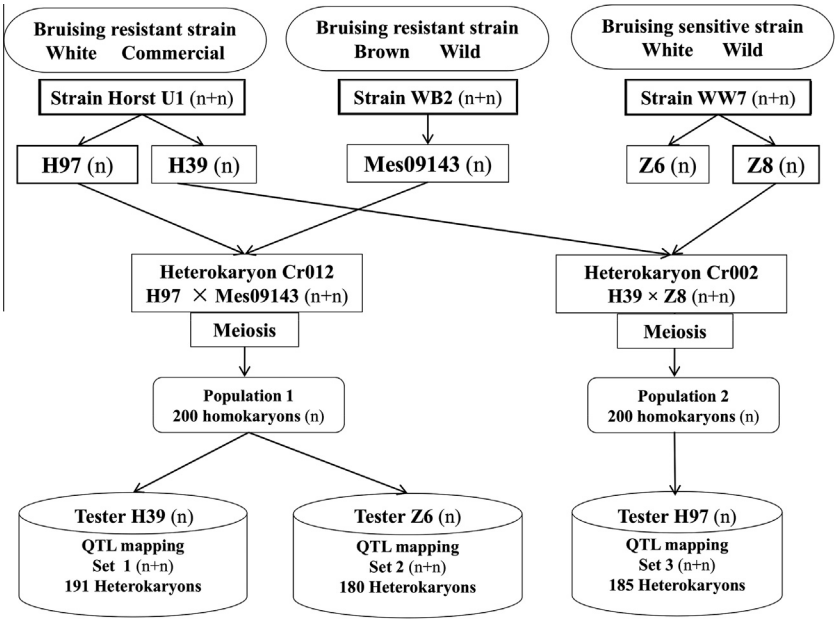

Fig. 1. Flow diagram to illustrate the generation of the three segregating populations used in this study. Here we use "*n*" and "*n* + *n*" to indicate the haploidy and diploidy level of lines in the pedigree. Since the two constituent nuclei of heterokaryon stay side by side in the mycelium cell but not fuse, "*n* + *n*" is used to indicate the diploid instead of "2*n*".

heterokaryon Set 2. Population 2 (offspring of H39 × Z8) was crossed with H97 to generate heterokaryon Set 3 (Fig. 1). Crosses were identified to be real heterokaryons as previously described (Gao et al., 2013). The three sets of heterokaryons were cultivated and used to analyze QTLs for bruising sensitivity and cap color. Not all the heterokaryons produced mushrooms. As a result, the number of fruiting individuals of the three sets of heterokaryons are 191, 180 and 185 respectively. The genotypic and phenotypic information of these individuals were used for genetic linkage mapping and QTL mapping in this study. The three original heterokaryotic lines (Horst U1, WB2, and WW7), and the two F1 hybrids (Cr012 and Cr002) were also included in the cultivation trials.

2.2. Cultivation tests and phenotypic measurements

One independent cultivation trial were carried out for each set of heterokaryons at the mushroom farm of Unifarm in Wageningen UR with controlled climate. Mushrooms were grown on commercial compost (CNC Substrates), spawned in 0.1 m^2 boxes (40 × 30 × 21 cm) filled with 8 kg of compost. Each individual was grown once in one box. Mushrooms were taken for analysis at the production peak of flush 1 and 2. For each set of heterokaryons, 20 mushrooms of each individual were measured for bruising sensitivity at 1 h after bruising as described in a previous study (Weijn et al., 2012). Bruising sensitivity (BS) and cap color (CC) were measured with the same computer image system. BS was measured as the difference of whiteness index between the control (non-bruised) area and the bruised area on the same mushroom, and CC was the whiteness index of the non-bruised area. In order to make the description clear, BS value of flush 1 mushrooms was coded as BS1; that of flush 2 was coded as BS2; similarly, CC data at two flushes were coded as CC1 and CC2. The three sets of heterokaryons were named as Set 1, 2 and 3.

2.3. Statistical analysis

Statistical analyses were performed in SPSS (IBM statistics 19) and Genstat (Version 15). Untransformed data were used for the summary statistics. Before applying analysis of variance (ANOVA), data were transformed using the square root (SQRT) function to fit a normal distribution. ANOVA was performed with data of each flush independently for the genotypic variation, according to the following model: $Y = \mu + G + \varepsilon$, where μ is the mean value, G is the genotypic effect, and ε is the residual effect. Data of heterokaryon Set 1 and Set 2 were used independently for ANOVA to estimate the flush effect with the following model $Y = \mu + G + F + G \times F + \varepsilon$. Flush 1 mushrooms of Set 1 and Set 2 were used for ANOVA to estimate the effect of tester lines with a model $Y = \mu + G + T + G \times T + \varepsilon$. In these ANOVA models, G represents genotypic effect; F represents flush effect, and T the tester line; $G \times F$ the genotype and flush interaction, $G \times T$ the genotype and tester line interaction; ε is the residual. The multiple comparison was performed according to the Student–Newman–Keuls (SNK) test. Transgression of BS and CC were determined by comparing the three sets of heterokaryons to their respective original heterokaryons.

Broad-sense heritability (H^2) was calculated for samples of each flush with a model $H^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_e^2/r)]$, for samples across flushes (flush 1 and flush 2) with a model $H^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_e G \times F^2/nr) + (\sigma_e^2/r)]$, and for data across tester lines (Set 1 and Set 2) with a model $H^2 = \sigma_G^2/[\sigma_G^2 + (\sigma_{G\times T}^2/nr) + (\sigma_e^2/r)]$, where σ_G^2 represented the genetic variance, σ_e^2 was the error variance (mean square of residual), $\sigma_{G\times F}^2$ was the variance of genotype and flush interaction, $\sigma_{G\times T}^2$ was the variance of genotype by tester line interaction. In this study, n was the number of flushes ($n = 2$) or tester lines ($n = 2$), r was the number of replicates within the experiment ($r = 1$).

2.4. Genetic linkage maps

The detailed segregation analysis and the construction of the two genetic linkage maps will be reported in another study (Gao et al., unpublished). Briefly, SNP markers were selected to be evenly distributed across the chromosome (see maps in Section 3) based on the genome sequences of the reference homokaryon H97 (http://genome.jgi-psf.org/Agabi_varbisH97_2/Agabi_varbisH97_2.home.html) (Morin et al., 2012). Although 200 homokaryons were generated for each segregating population,

191 fruiting genotypes of population 1 and 180 fruiting genotypes of populations 2 were used for genetic linkage mapping. The total map length of population 1 (heterokaryon Set 1 and Set 2) was 164 cM generated with 95 SNP markers. It has an average crossover frequency of 0.1 per individual per chromosome. The total map length of population 2 (heterokaryon Set 3) was 86 cM generated with 76 SNP markers, and it has an average crossover frequency of 0.05 per individual per chromosome.

2.5. QTL detection

QTL detection with simple interval mapping (SIM) and composite interval mapping (CIM) was done in Genstat (Version 15) with the model of the single trait (single environment) QTL. After the initial scan (SIM), the loci having a test statistic larger than the threshold ($-\log10(P)$) were detected, and the ones having the largest $-\log10(P)$ on each chromosome were selected as co-factors for CIM. Several rounds of CIM were done until no new QTLs were detected. The phenotypic variation explained (explained variance: EV %) by each QTL and the additive effects of each QTL were given by this model. The total phenotypic variation (total EV %) explained by all detected QTL was determined by multiple-regression analysis using markers closest to the highest $-\log10(P)$ for each putative QTL (Foulongne-Oriol et al., 2012).

QTL mapping was done for both BS and CC of the three sets of heterokaryons. Next to it, heterokaryon Set 1 and Set 2 were divided into two groups according to the cap color, i.e., a non-white group (off-white, light brown and brown) and a white group. QTL mapping for BS was done for both groups separately in order to avoid possible interference from cap color. Only results from the composite interval mapping are shown in this paper. Because of the low resolution of the genetic linkage map QTLs on the same chromosome were considered as one QTL, which were named after the chromosome number.

3. Results

3.1. Statistical analysis of BS and CC of population 1 (heterokaryon Set 1 and 2)

A summary statistics of BS and CC was done for the original heterokaryotic lines (Horst U1 and WB2), the hybrid Cr012 (used to generate population 1) and heterokaryons of Set 1 and Set 2 (Supplementary Table 1). Data of each flush were analyzed independently. Heterokaryotic lines WB2 and Horst U1 were both less sensitive to bruising than the sensitive line WW7 as found in previous tests (Weijn et al., 2012), and WB2 was significantly less sensitive to bruising than Horst U1 in both flush 1 and flush 2 ($P < 0.05$). BS value of the hybrid heterokaryon Cr012 (Mes09143 × H97) was in most cases near the value of the mid-parent (mean of the two original heterokaryons). Since WB2 is brown and Horst U1 is white the CC value (whiteness index of cap) of WB2 is much lower than Horst U1. The standard deviation of CC-WB2 was higher than that of CC-Horst U1 indicating a large variation in cap color of this brown strain. The cap color of the hybrid (Cr012) was on average close to its brown parent WB2, which indicated dominance of the brown allele as found in previous studies (Callac et al., 1998). The average BS value of heterokaryon Set 2 was significantly higher (more sensitive) than that of heterokaryon Set 1, and the average CC value of heterokaryons of Set 2 is lower (darker) than that of heterokaryons of Set 1 ($P < 0.05$). This indicates the significant influence of different genetic background (tester line) on the phenotypic expression of BS and CC.

The continuous distribution of BS indicates its polygenic feature (Fig. 2). A typical bimodal distribution was observed for CC (Fig. 3), which was consistent with the fact that cap color, i.e., white or non-white, was indeed mainly controlled by one locus. Clearly two groups are present, i.e., non-white group with large variation of CC, and white-group with smaller variation. To a lesser extent, also a bimodal distribution of BS was found (e.g. BS2 in Fig. 2). Specifically, the non-white individuals of Set 1 and Set 2 were less sensitive to bruising than the white individuals indicating the possible co-segregation of loci for BS and CC.

Transgression in the offspring was found for both BS and CC in Set 1 and Set 2 using multiple comparison (SNK test, $P < 0.05$). Transgression for BS (individuals more sensitive than the original heterokaryotic lines) was found in both Set 1 and Set 2 in both flushes, but transgression for bruising resistance (BR, individuals more resistant than WB2) was found only with data of BS2 of Set 1. No transgression for BR was found in Set2, which might be due to the high bruising sensitivity level of the tester line Z6. The general mushroom quality of Set 2 was bad showing early maturation, low firmness, and irregular appearance (data not shown) indicating the influence of the tester line Z6 that was derived from a low-quality line. In both Set 1 and Set 2 transgression for brown cap (color) was found in the two flushes, i.e., some progeny had a significantly darker cap color than the brown heterokaryotic line WB2. Transgression for white cap (color) was found in Set 1 and Set 2 (flush 1), i.e., some individuals of Set 1 and Set 2 were whiter than the white heterokaryotic line Horst U1.

Set 1 and Set 2 were split into two groups (non-white and white) based on the observation of flush 1 of Set 1 (Supplementary Table 1). The mean BS of the non-white group was significantly lower than that of the white group. As expected, the mean CC value of the non-white group was significantly lower than that of the white group ($P < 0.001$). The standard deviation (SD) of the CC-non-white was larger than that of CC-white, indicating the larger variation of cap color in non-white mushrooms compared to white mushrooms (SD is shown in parentheses in Supplementary Table 1). The average cap color for Set 1 and Set 2 also differed, obviously caused by the different testers (H39 and Z6) used. That resulted in a shift for some individuals from white in Set 1 to off-white in Set 2.

Results of ANOVA showed that the genotype was a significant factor for both BS and CC ($\alpha = 0.001$). Two-way ANOVA to test the flush effect was performed with SQRT of BS and CC. Untransformed data were used for ANOVA to test the effect of tester lines. BS and CC were also significantly influenced by flushes and tester lines (across Set 1 and Set 2) ($\alpha = 0.001$). In average, BS2 was significantly lower than that of BS1; the mean BS of Set 1 (population 1 crossed with tester H39) was significantly lower than that of Set 2 (population 1 crossed with tester Z6). The interaction effects of genotype by flushes and genotype by tester lines were both significant ($\alpha = 0.001$), indicating that genotypes react differently with respect to these factors and the interaction effects should be taken into account when assessing BS and CC. Broad-sense heritability (H^2) was high for both BS and CC and also across flushes and tester lines. H^2 was 0.88 for BS across flushes and 0.88 for BS across tester lines; H^2 was 0.96 for CC across flushes, and it ranged from 0.95 to 0.99 for CC across tester lines. The high heritability indicated that BS and CC are both highly inheritable traits, and genotypic effect was the main effect to generate phenotypic variations for BS and CC.

Correlation of data across flushes (BS1 and BS2) was tested with Spearman's rank correlation. For both BS and CC, data across flushes were highly correlated. The correlation coefficients between CC1 and CC2 were higher compared to those between BS1 and BS2. The correlation of BS or CC across two tester lines (H39 and Z6) was tested based on the mean value of BS1 and

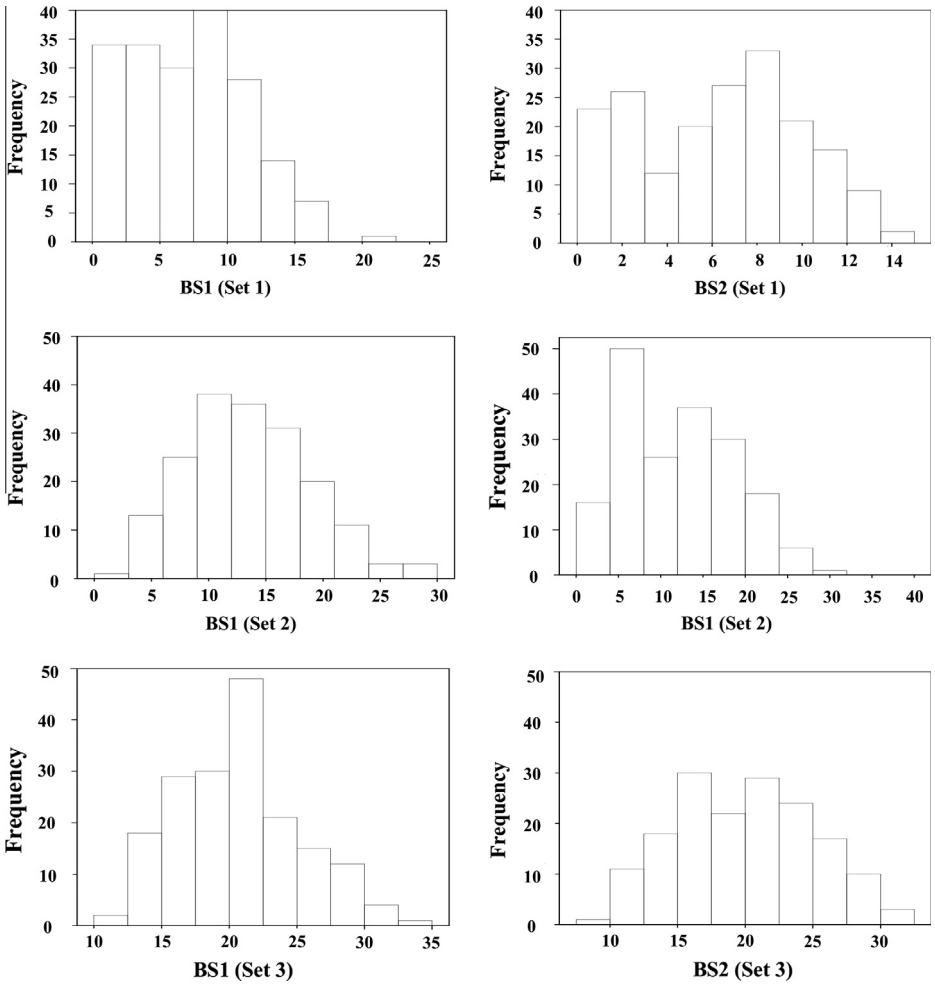

Fig. 2. Frequency distribution of bruising sensitivity of the three heterokaryon sets BS1 for the bruising sensitivity of flush 1 BS2 for the bruising sensitivity of flush 2; the horizontal axis indicates value range of BS, and the vertical axis indicates the frequency of individuals.

CC1 (sample size: 180). BS1-Set 1 and BS1-Set 2 were significantly correlated ($r = 0.70$, $\alpha = 0.001$), and CC1-Set 1 and CC1-Set 2 also highly correlated ($r = 0.77$, $\alpha = 0.001$). The high correlation of data underlines the consistency in phenotyping BS and CC in this experimental setup.

3.2. Statistical analysis of BS and CC of population 2 (heterokaryon Set 3)

The two original heterokaryotic lines (Horst U1 and WW7) showed significant difference in both BS and CC ($P < 0.001$) (Supplementary Table 2). WW7 was much more sensitive to bruising than Horst U1, and WW7 was less white than Horst U1. The BS of hybrid heterokaryon Cr002 was intermediate compared to its two original heterokaryotic lines (Horst U1 and WW7), and CC of the heterokaryon Cr002 was generally close to the mid-parent as well. No transgression was found in heterokaryon Set 3 for BS, while transgression was found for CC, i.e., some individuals were significantly whiter than Horst U1 ($P < 0.05$). The continuous distribution for both BS and CC of Set 3 indicates the polygenic features for both BS and CC (Figs. 2 and 3).

One-way ANOVA was performed with BS and CC of the two flushes respectively. Two-way ANOVA was performed across flushes. Significant genotypic effects were observed for both BS and CC ($\alpha = 0.001$). BS and CC were significantly influenced by flushes ($\alpha = 0.001$); the interactions of genotype by flushes were significant as well ($\alpha = 0.001$).

Broad-sense heritability (H^2) was high for both BS and CC across flushes, which was 0.89 for BS to 0.80 for CC. The high heritability in Set 3 indicates again that BS and CC are highly inheritable traits, and the genotypic effects are the main effects to generate phenotypic variations for BS and CC. BS1 and BS2 were highly correlated as where CC1 and CC2. The correlation coefficient (r) of BS across flushes was generally higher compared to that of CC.

3.3. QTL detection in population 1 (heterokaryon Set 1 and 2)

3.3.1. QTL mapping for bruising sensitivity (population 1)

Since heterokaryon Set 1 and 2 were generated from population 1 crossed with two different tester lines, results of Set 1 and Set 2 are presented together. With the availability of the genome sequence and the physical positions of SNP markers, the linkage group of the genetic map in this study is equivalent to chromosomes, i.e., the linkage group is numbered after the chromosome number (Foulongne-Oriol et al., 2010). Because of the short genetic linkage map (due to the low recombination frequency), the confidence interval for most of the QTL almost cover whole chromosomes apart from chromosome 8 where crossovers were seen more frequently (Fig. 4). An average $-\log(P)$ threshold of 2.8 was used to declare a significant QTL.

Across data of the two heterokaryons sets of population 1, a total of 9 QTL were detected, with the individual EV % ranging from 1.39% (BS1-Set 1 on chromosome 12) to 71.2% (BS1-Set 1 on chromosome 8). The total phenotypic variation explained by all QTL

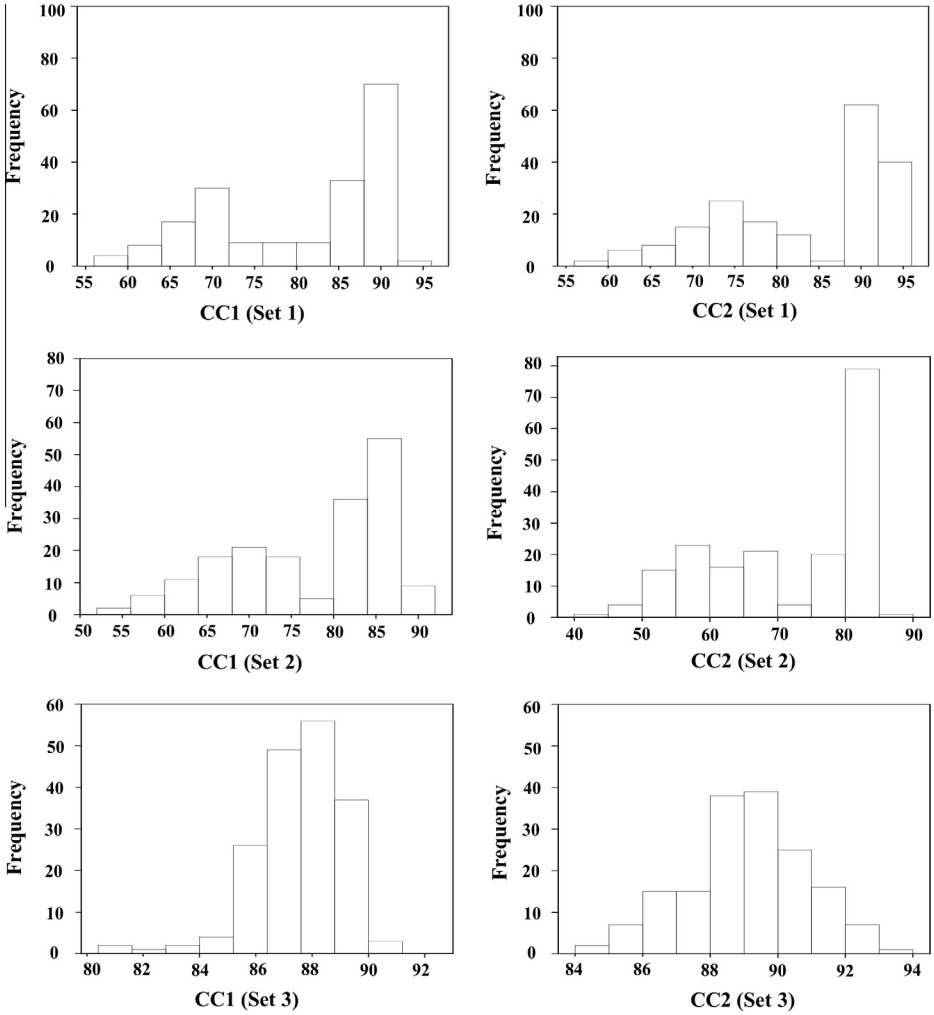

Fig. 3. Frequency distribution of cap color of the three heterokaryon sets CC1 for the cap color of flush1 and CC2 the cap color of flush 2; the horizontal axis indicates value range of CC, and the vertical axis indicates the frequency of individuals.

(total EV %) ranged from 60.7% for BS1-Set 2 to 77.6% for BS1-Set 1. Across data of two flushes, common QTL for BS were detected on chromosome 3, 8 and 10 in Set 1 and on chromosome 7, 8 and 10 in Set 2 (see Supplementary Tables 3 and 4). The major QTL for BS was on chromosome 8 in both Set 1 and Set 2. In Set 1 it explained 71% BS variation in flush 1 and 64% BS variation in flush 2; in Set 2 it explained 44% BS variation in flush 1 and 68% of that in flush 2. The high value (more sensitive to bruising) allele of the QTL on chromosome 8 was from H97, i.e., the beneficial allele of this QTL for low bruising sensitivity was from Mes09143. The high value allele of QTLs on chromosome 7 was also from H97, while the high value alleles of QTL on chromosome 3 and chromosome 10 were consistently from Mes09143. The differences in QTL detected for Set 1 and Set 2 indicate the impact of tester lines on phenotype expression. Some QTL are detected either only in flush 1 or in flush 2. For example, apart from the three consistent QTL on chromosome 3, 8 and 10 in heterokaryon Set 1, three extra QTL on chromosome 1, 6 and 12 were specific for BS1 but not for BS2. In addition to the three consistent QTL on chromosome 7, 8, 10 detected in heterokaryon Set 2 a QTL on chromosome 9 was found for BS2 but not for BS1.

BS was assessed by measuring the discoloration of the bruised area relative to the background (non-bruised area on the same mushroom). Although this will correct for the cap color of the mushroom it is possible that the cap color interferes (Fig. 4). Set 1 and Set 2 were, therefore, split up into a non-white and a white group based on the cap color of heterokaryon Set 1 (flush 1). As expected, the major QTL on chromosome 8 disappeared, while the minor QTLs detected in the whole set of heterokaryons became more significant in the two groups.

3.3.1.1. QTL mapping for BS in subpopulations of Set 1. For Set 1, consistent to the QTL mapping with the whole set of heterokaryons, QTL on chromosome 3 and chromosome 10 were detected in the white group of flush 1 and in both groups of flush 2 (Supplementary Table 3). However, consistent QTL for both non-white and white group of flush 1 were the ones on chromosome 1 and chromosome 3; consistent QTLs for both non-white and white groups of flush 2 were located on chromosome 3 and chromosome 10. In addition, QTL on chromosome 6 was specific for non-white group of flush 1, and chromosome 9 and chromosome 12 were specific for white group of flush 1. The most significant QTL in the split data set was on chromosome 10 with the explained variance up to 34% for white group of flush 2. The high value alleles (more sensitive to bruising) of QTL on chromosome 3, 9, and 10 were from the parental homokaryon Mes09143, and those on chromosome 1, 2, 6 and 12 were from the parental homokaryon H97.

3.3.1.2. QTL mapping for BS in subpopulations of Set 2. In Set 2, minor (non chromosome 8) QTL on chromosome 3 and 7 were detected in

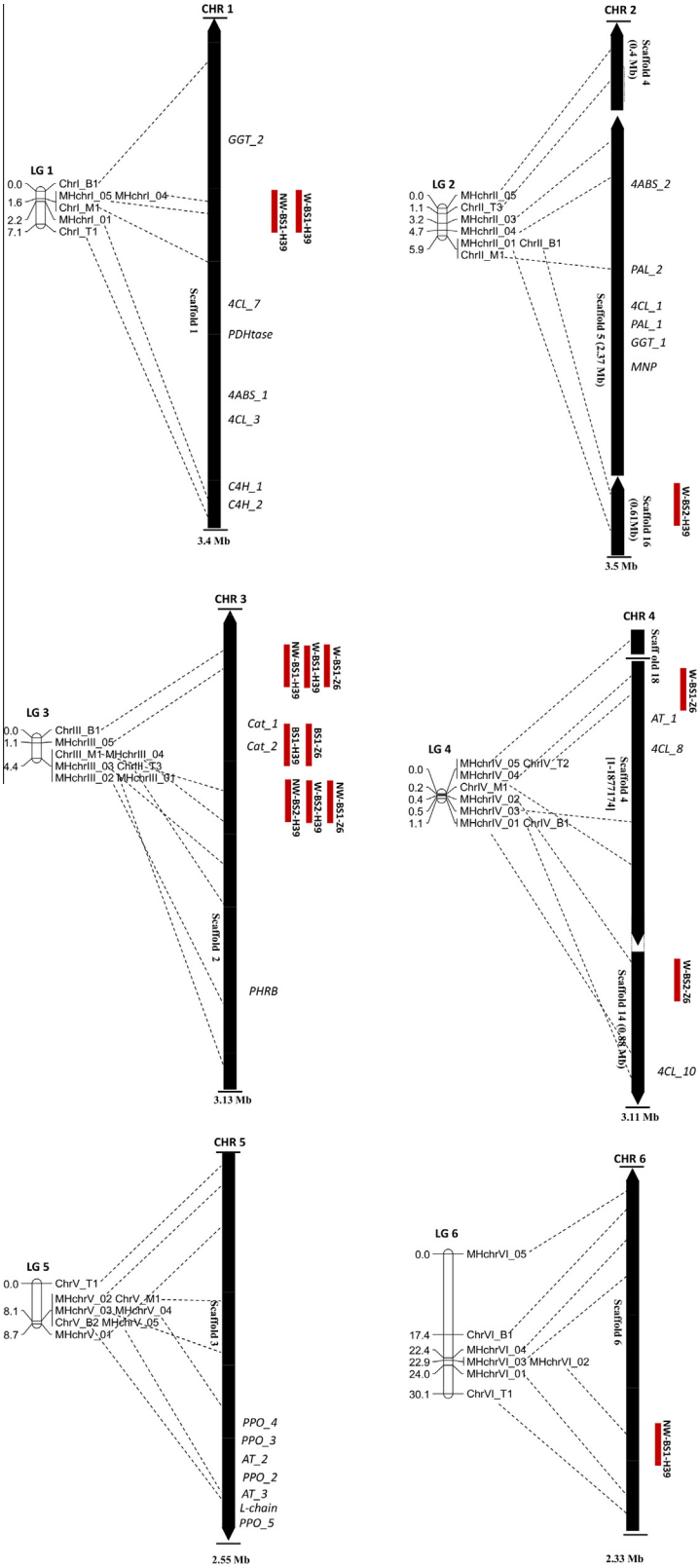

Fig. 4. Genetic linkage map and QTL locations for bruising sensitivity of population 1. Physical maps are presented as black bars and coded as chromosome (1–13) in order to clarify the position of each SNP marker. Linkage groups are coded as LG. Marker names are provided on the right side of each linkage group; the genetic positions are shown on the left side; Markers and their approximately physical positions are linked with dotted lines. The physical positions of SNP markers were determined by the genome sequence of H97 (http://genome.jgi-psf.org/Agabi_varbisH97_2/Agabi_varbisH97_2.home.html). QTL with explained variance higher than 5% are plotted here with short bars on the right side of the physical map. Since the very low recombination frequency and a short map the threshold of the QTL mostly covers the whole genetic linkage map, the length of QTL bars do not represent their confidence intervals. QTL bars are positioned nearby the physical positions of the linked markers except for chromosome 8. The bar length of the ones on chromosome 8 indicates the upper and lower bound of QTL. A total of 42 genes identified previously that are involved in the melanin biosynthesis pathway (Weijn et al., 2013a) were plotted in italic font on the right side of the physical map based on their approximate positions on the scaffold. The major QTL for cap color are overlapped with the QTL of bruising sensitivity on chromosome 8, which were not plotted in this figure.

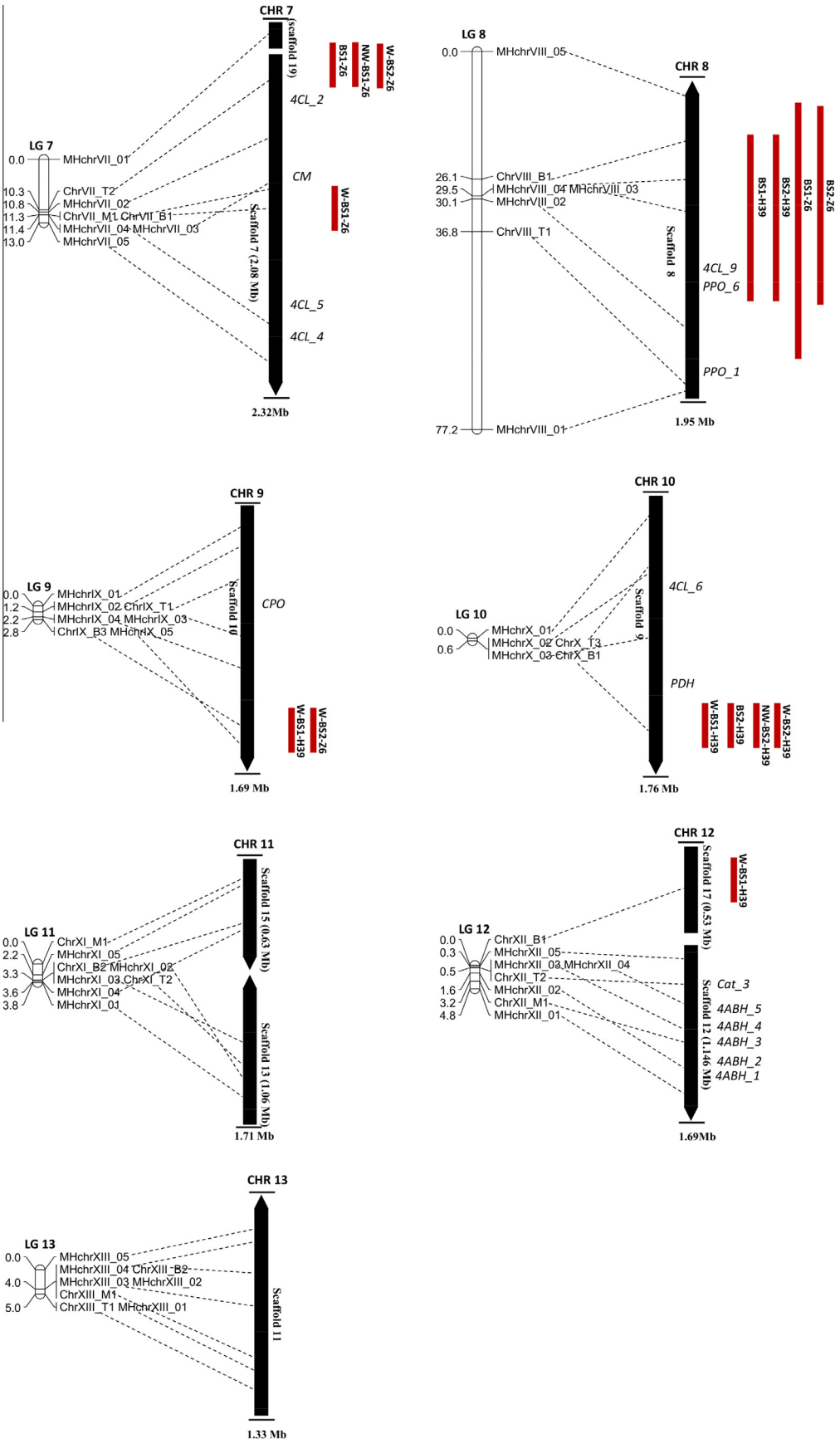

Fig. 4 (*continued*)

both non-white and white group of flush 1; QTL on chromosome 4, 7 and 9 were detected in the white group of flush 2, but no QTLs were detected in the non-white group of flush 2 (Supplementary Table 4). Chromosome 7 was the consistent and most significant minor QTL over two flushes of Set 2, and the explained variance was up to 25% in the white group of flush 2. The high value alleles of chromosome 7 were consistently from the parental homokaryon H97. Fewer QTLs were found in the two subpopulations (non-white and white) compared to the whole set of heterokaryons. For instance, no QTL was detected in non-white group of flush 2, and the one on chromosome 10 detected in the whole set of heterokaryons was not detected in either non-white or white group. This might be due to the small subpopulation size, and the phenotypic variation was not big enough to detect the marker–trait association. Nevertheless, there are still extra QTLs found in both subpopulations but not in the whole heterokaryon Set 2. The one on chromosome 4 was detected with the white-BS1 but not with BS1. In summary, QTLs on chromosome 3 and chromosome 7 were generally consistent for both Set 2 heterokaryons and subpopulations.

In conclusion, chromosome 8 is the major QTL for BS for the whole heterokaryon Set 1 and Set 2. Chromosome 8 also contains the major QTL for cap color (see next paragraph). Due to the low recombination frequency, a sorting for color also leads to sorting for this QTL and eliminates segregation for the QTL on chromosome 8 in white and non-white groups separately. As a result, the significance of the other QTLs, which was minor in the whole set of heterokaryons, increased in significance in the two subpopulations. The high value alleles for all the QTL of BS were consistent in Set 1 and Set 2. In summary, chromosome 3, 9 and 10 of H97 are beneficial alleles for bruising resistance, and Mes09143 has beneficial alleles on chromosome 1, 2, 4, 6, 7, 8 and 12 for resistance to bruising.

3.3.2. QTL mapping for cap color (population 1)

A major QTL for cap color was found on chromosome 8 in population 1 with both tester lines. It has an extremely high EV % for the phenotype, which varied from 77% to 85%. This is in agreement with an earlier study (Foulongne-Oriol et al., 2010). Since this QTL overlaps with the major QTL of BS, it was not plotted again in Fig. 4. One marker named MHchrVIII_03 was linked with the QTL, and the map region on chromosome 8 was from 14.5 cM to 44.5 cM (Fig. 4). Compared to the physical region, two relevant genes involved in the melanin biosynthesis pathway were located in this region, i.e., *PPO6* and *4CL_9*, which were identified in the genome of *A. bisporus* (Weijn et al., 2013a). Identification of the *PPO6* and *4CL_9* within the QTL region suggests that these two genes may be candidate QTL genes involved in the mechanisms of cap color and/or bruising sensitivity that is worthwhile to further investigate.

Apart from this major QTL, minor QTLs were found on chromosome 1, 2, 3, 4, 5, 7, 10, and locations of the QTL varied between different flushes and heterokaryon sets (Supplementary Table 5). Only the QTL on chromosome 5 was a consistent one in both Set 1 and Set 2. The $-\log(P)$ values of most minor QTL were just above the threshold, and they explained less than 3% of the CC variation. The total phenotypic variation explained by all QTL reached to 87.9% for CC2-Set 2. The high value (white) allele of the major QTL on chromosome 8 was, as expected, from the H97 (derived from the white hybrid), while the high value alleles of the other minor QTL were generally from the brown parent Mes09143. It is known that brown is dominant over white and thus the allele from the brown parent is the dominant one (Callac et al., 1998). The minor QTL might thus explain to a larger extent the variation in brown color seen in the non-white individuals of Set 1 and Set 2.

3.4. QTL detection in population 2 (heterokaryon Set 3)

3.4.1. QTL mapping for bruising sensitivity (population 2)

Since for the construction of heterokaryon Set 3 only homokaryons derived from white lines were used, only white mushrooms were produced. Across the two flushes, a total of 7 QTL on chromosome 1, 2, 3, 5, 7, 8, 10 were detected for BS (Supplementary Table 6, Fig. 5). The total phenotypic variation explained by all the QTL detected was 57.0% for BS1-Set 3 and 57.6% for BS2-Set 3. The ones on chromosome 1 and 2 were the two major and consistent QTL. The QTL on chromosome 1 had an explained variance of 16% for BS1 and 12% for BS2, and that on chromosome 2 explained 28% phenotypic variation of BS1 and 42% phenotypic variation of BS2. Two minor QTL on chromosome 7 and 8 were only found for flush 1, and three minor QTL on chromosome 3, 5 and 10 were specific for flush 2 (Supplementary Table 6). The high value BS alleles of all QTL were from the sensitive parental line Z8 indicating that alleles of this strain have a considerable undesirable effect on sensitivity to bruising. In contrast, alleles of H39 on chromosome 1 and 2 are major beneficial alleles for bruising resistance.

In summary, there is an overlap between QTL detected in the three sets of heterokaryons. QTL on chromosome 3, 8 and 10 were found in all three sets of heterokaryons. QTL were also shared by the white offspring in Set 1 and Set 3, i.e. the major QTL on chromosome 1 for white-BS1 and the one on chromosome 2 for white-BS2.

3.4.2. QTL mapping for cap color (population 2)

A total of 3 QTL on chromosome 2, 7, and 12 were detected for CC in heterokaryon Set 3 (Supplementary Table 7, Fig. 5). The phenotypic variation explained by all QTL reached 31.2% for CC1-Set 3. The high value (white) alleles of the QTL on chromosome 2 and 7 are both from the more white parent H39, but the other QTL on chromosome 12 have the high value alleles from the lesser white parent Z8. Consistent QTL for CC in Set 3 were located on chromosome 7, which explained 17% phenotypic variation of CC1 and 20% phenotypic variation of CC2. The two QTL on chromosome 2 and 7 were also detected in Set 1 and Set 2, in which also a QTL was detected on chromosome 2 with Set 1 data of both flushes and a QTL on chromosome 7 detected with CC1 in Set 2. However, the explained variance in Set 1 and Set 2 were much lower than that in Set 3. QTL for CC detected in Set 3 are relevant for retaining a white color or improving whiteness in breeding programs using only white varieties.

4. Discussion

Three heterokaryotic parents, differing in bruising sensitivity, were selected to generate two segregating populations and map QTL for bruising sensitivity and cap color with the purpose to provide a genetic basis for breeding button mushrooms that are resistant to mechanical bruising. To our knowledge, this is the first QTL analysis for bruising sensitivity of *A. bisporus* and also the first QTL analysis for cap whiteness in a white population (heterokaryon Set 3). Since reports on QTL analyses for edible fungi are scarce, this study contributes considerably to our knowledge on QTL analysis for edible fungi in general and to the understanding of complex traits in edible fungi.

QTL analyses for button mushroom have been done so far only in an intervarietal population of var. *bisporus* × var. *burnetti* (Foulongne-Oriol et al., 2010, 2012; Moquet et al., 1999) and not in an intravarietal population of var. *bisporus*. Since the recombination frequency in the *burnettii* variety seems to be normal, offspring of intervarietal heterokaryons generate linkage maps of high resolution. Here we used only lines from bisporic varieties

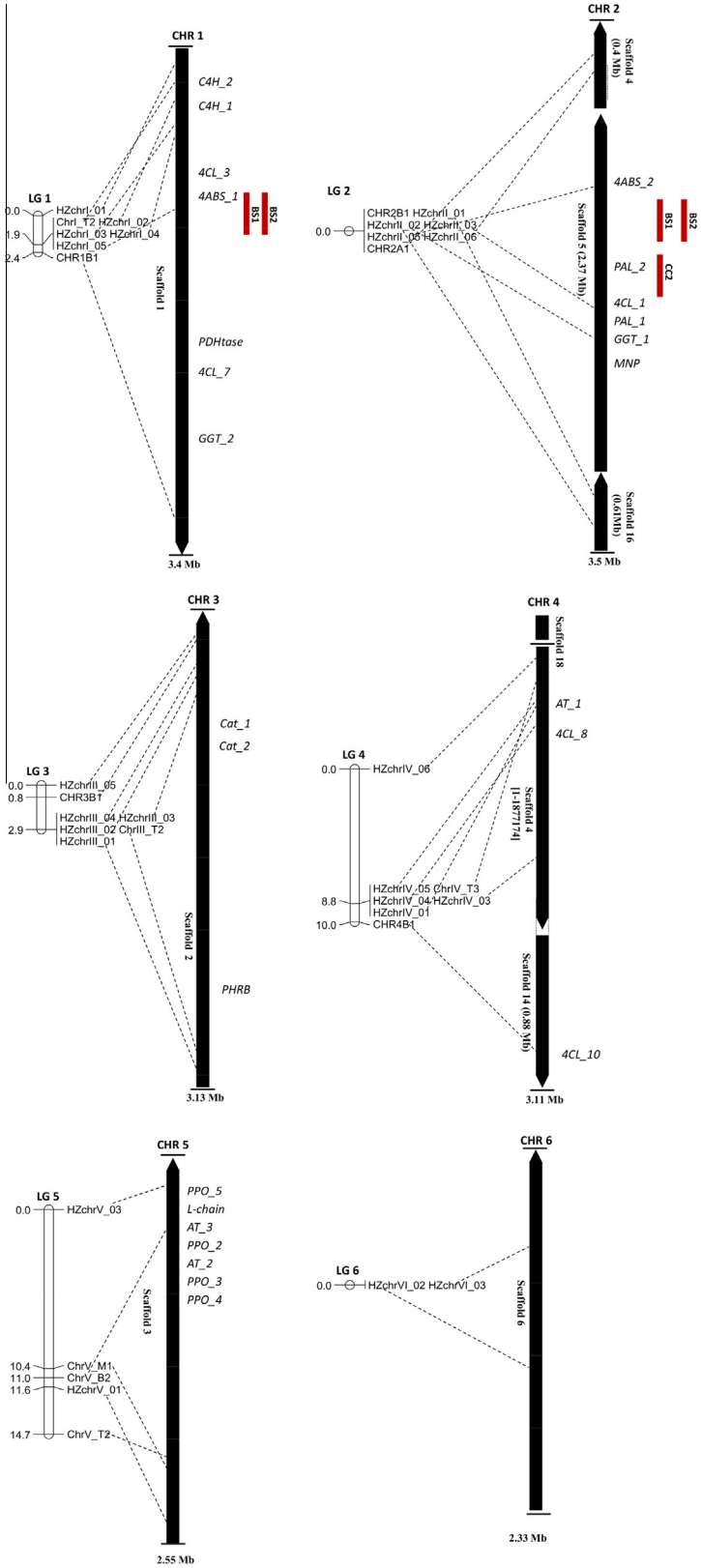

Fig. 5. Genetic linkage map and QTL locations for bruising sensitivity and cap color of population 2. Physical maps are presented as black bars and coded as chromosome (1–13) in order to clarify the position of each SNP marker. Linkage groups are coded as LG. Marker names are provided on the right side of each linkage group; the genetic positions are shown on the left side; Markers and their approximately physical positions are linked with dotted lines. The physical positions of SNP markers were determined by the genome sequence of H97 (http://genome.jgi-psf.org/Agabi_varbisH97_2/Agabi_varbisH97_2.home.html). QTL with explained variance higher than 5% are plotted here with short bars on the right side of the physical map. Since the very low recombination frequency and a short map the threshold of the QTL mostly covers the whole genetic linkage map, the length of QTL bars do not indicate their confidence intervals. QTL bars are positioned nearby the physical positions of the linked markers. A total of 42 genes identified previously that are involved in the melanin biosynthesis pathway (Weijn et al., 2013a) were plotted in italic font on the right side of the physical map based on their approximate positions on the scaffold.

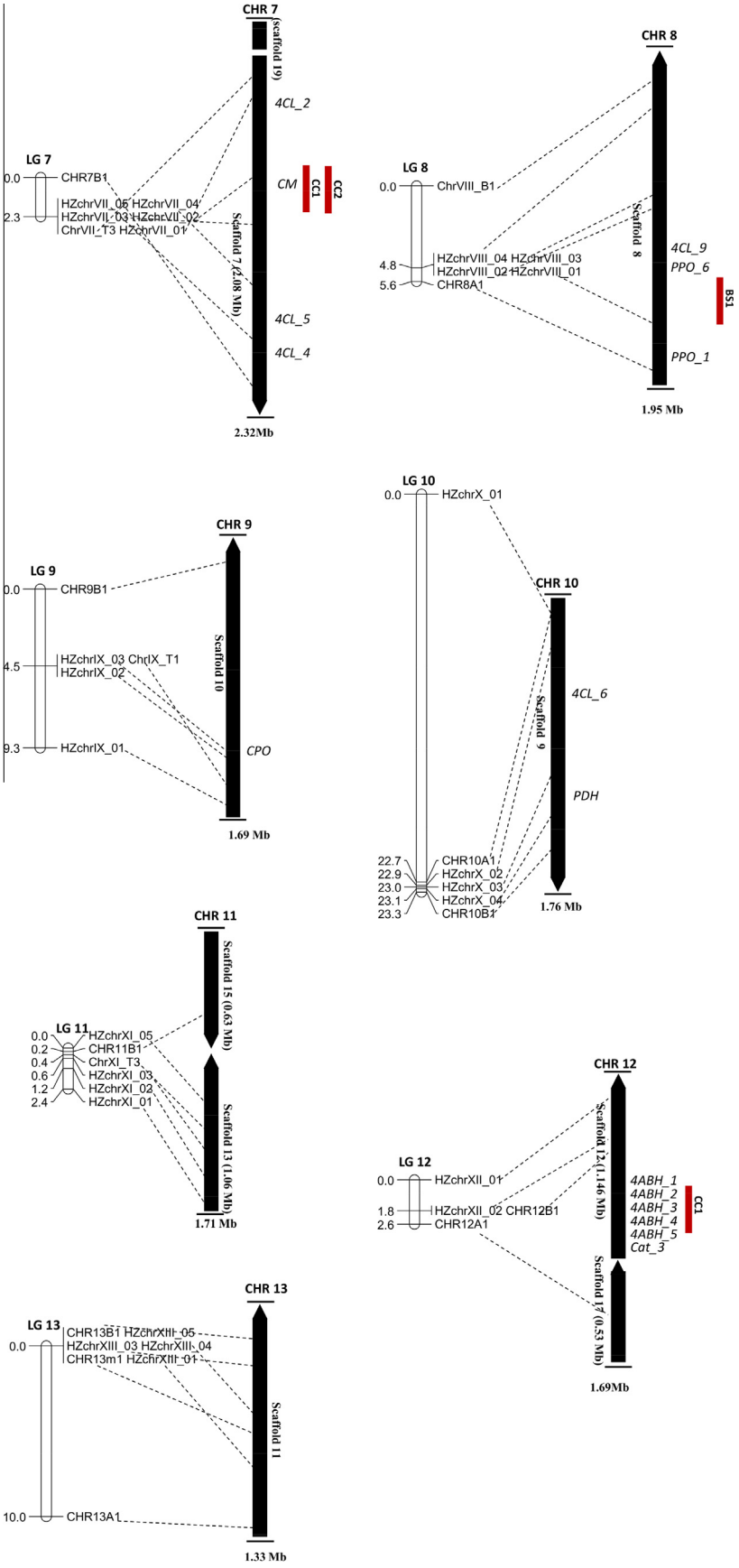

Fig. 5 (*continued*)

because of the poor quality (including bruising sensitivity) of the tetra-sporic lines we have tested so far. The recombination frequency is low in all bisporic varieties here between SNP markers spread evenly over the entire chromosome region, which is presented as well in previous studies (Kerrigan et al., 1993) (Figs. 4 and 5). A large number of SNP markers could be selected for genetic linkage mapping with the availability of the whole genome sequence of H97 and the re-sequencing of the other parental homokaryons, but it is not helpful for our study to construct a saturated linkage map because of the low recombination frequency. Less markers were thus selected. This resulted in linkage maps with a very low resolution and QTL can thus only be assigned to chromosomes. Mapping genes involved in controlling normal recombination frequency and introducing these genes in breeding stocks will be needed to improve breeding efficiency in the button mushroom.

In order to increase the accuracy of QTL detection, two different segregating populations of homokaryons were generated for QTL mapping. For mapping bruising sensitivity, fruiting bodies are required, and in order to obtain these, homokaryons are mated with a compatible homokaryon (a tester homokaryon) to restore the heterokaryotic phase. For each locus, these mushrooms (F2) thus contain either the allele from parental homokaryon 1 or parental homokaryon 2 in one nucleus and all contain the same allele from the tester line in the other nucleus. In this way alleles of a segregating homokaryotic offspring are mapped in a shared genetic background of the tester line. The use of different segregating populations and different tester lines allows the study of traits in different genetic backgrounds, which indeed had a large impact on the phenotype and this influenced the magnitude of QTL and to some extent the location. The interaction effect of genotype by tester lines is significant as previously reported (Larraya et al., 2002). This led to the inconsistencies in QTL detection for the two sets of heterokaryons of population 1. Therefore, minor QTL detected for only one heterokaryon set might not be sufficient to reveal significant marker–trait association (Foulongne-Oriol et al., 2012).

The two parental homokaryons of population 1 both originate from resistant heterokaryons. The beneficial alleles of QTL detected for resistance to bruising were indeed found in both parental lines with both tester lines. The two parental homokaryons of population 2 originate from a resistant heterokaryon (Horst U1) and a sensitive heterokaryon (WW7). The beneficial alleles of QTL for bruising resistance in heterokaryon Set 3 were detected only in the resistant parent H39. This consistency strengthens the QTL results in our study. The two lines H97 and H39 used in this study are the two constituent homokaryons of the commercial line Horst U1. Like all present-day commercial lines, Horst U1 has a certain resistance to bruising but the level of resistance is not enough to allow mechanical harvest for the fresh market. The purpose of this study was, therefore, to map the beneficial alleles underlying the QTL in both constituent homokaryons of Horst U1 and in one of the constituent homokaryons (Mes09143) derived from a more resistant line WB2. This can be used to retain the bruising resistance in Horst U1 and add additional resistance from a more resistant strain in an introgression breeding program. By crossing population 1 (offspring of H97 × Mes09143) with tester homokaryon H39, the beneficial alleles of the three resistant homokaryons (Mes09143, H97, and H39) can be stacked in one or more individuals of heterokaryon Set 1. Many heterokaryons of Set 1 were indeed more resistant to bruising than Horst U1 suggesting that the beneficial QTL of WB2 (Mes09143) were introduced in a Horst U1 background.

In the space-limited cultivation facility, we used the maximum number of genotypes without replications (instead of less genotypes in replicates) to generate as much useful data as possible. Each individual (genotype) was thus grown once in one box, which resulted in the weakness of the statistical power of the analyses. Nevertheless, the measurements of bruising sensitivity in flush 1 and flush 2 were used for QTL mapping. Major QTL remained the same for flush 1 and flush 2, while some minor QTL were specific for one flush, which is in agreement with the fact that flush has significant effects on bruising sensitivity based on ANOVA. Flush specific QTL might also indicate that the different compost or casing conditions in flush 1 and 2 influence the trait and cause significant genotype by environment (QTL by flush) interaction.

The major BS-QTL overlapped with the CC-QTL on chromosome 8, which could be seen from the similar bimodal distribution of the two traits in Set 1 and Set 2. With cap color as a grouping factor, bruising sensitivity of the white group is significantly higher than that of the non-white group, which is in agreement with that reported in previous studies (Gao et al., 2013). Due to the low recombination frequency it is difficult to say if the QTL for cap color and bruising sensitivity represent the same genes with pleiotropic effects or closely linked genes. Since a minor QTL for BS of population 2 (Set 3) was also detected on chromosome 8, but no QTL was found for cap color on the same chromosome, the major brown allele is thus for the most part responsible for the difference in bruising sensitivity between brown and white individuals, but not for the differences among white individuals. If the QTL on chromosome 8 represents a major gene for cap color and for bruising sensitivity, it will be impossible to use this gene to generate bruising insensitive white varieties. The same accounts for the QTLs of the disease resistance against *P. tolaasii* also causing browning. Moquet et al. (1999) showed that resistance to this bacterial blotch was also linked to cap color and suggested that mechanisms of melanin biosynthesis for natural (cap color) or pathogenic browning are strongly linked (Moquet et al., 1999). If these phenomena represent different linked genes, varieties with normal recombination have to be used as intermediate to unlink these genes to break the linkage drag.

According to the melanin biosynthesis pathway, a number of enzymes (42 genes identified in the genome, Fig. 4) and phenolic compounds are involved in the mechanism of the bruised-induced discoloration (Weijn et al., 2013a, 2013b). The level of gene expression producing these enzymes and phenolic compounds are expected to be associated with the degree of bruising sensitivity. Thus, next to the QTL analysis for bruising sensitivity, several individuals of heterokaryon Set 1 were selected and analyzed for expression of the genes involved in the melanin biosynthesis pathway and the synthesis of phenolic compounds present in cap skin tissue (Weijn, 2013). For most genes analyzed, no different expression was found between resistant and sensitive offspring. Only small differences in the expression level of three genes on chromosome 5, i.e., *L-chain*, *PPO_3* and *PPO_5*, were observed among several non-white individuals (Weijn, 2013). For chromosome 5, however, no major QTL was detected that associate with bruising sensitivity. A high correlation was found between the total concentration of phenolic compounds and bruising sensitivity for the white individuals, but this correlation was not seen among non-white individuals. The lack of correlation between levels of gene expression and differences in bruising sensitivity indicates that other, so far undetected, mechanisms are involved.

The bimodal distribution of cap color in heterokaryon Set 1 and Set 2 indicates that one major gene is responsible for cap color. Similar bimodal distributions for cap color were shown in previous studies (Foulongne-Oriol et al., 2012). Although in one of the previous papers cap color was considered as a dominant trait (Callac et al., 1998), we often see in our breeding programs an incomplete dominance since a cross between homokaryons derived from a brown and a white line usually produce mushrooms with a lighter color than the brown line. The cap color of the F1 heterokaryons used to generate population 1 is close to the brown original line

WB2. Since we could only recover one homokaryon (Mes09143) of this brown resistant line (WB2) we do not know the color allele of the other constituent nucleus and cannot conclude if the color close to the original brown line means complete or incomplete dominance. The selection for major QTL for cap color separates the progeny in non-white and white as found in previous studies (Callac et al., 1998; Foulongne-Oriol et al., 2012). Major alleles are often accompanied with modifier alleles. Modifier alleles for cap color were found on several other chromosomes in population 1 and they are likely responsible for the cap color ranging from brown to very light brown. However, a QTL on chromosome 13 reported previously (Foulongne-Oriol et al., 2012) were not found in our study. The large variation within the non-white group indicates that the effect of this major locus can be deflated by some modifier loci generating varied cap color. Based on data from population 1, it is difficult to conclude whether these modifier loci also determine the variation in whiteness of mushroom cap in a white population. The cap color of progeny of a cream (off-white) × white cross showed variations and was shown to be a polygenic trait instead of a single gene controlled trait (Miller et al., 1974). Major QTL for cap whiteness of population 2 were detected as well in population 1, which strengthens our conclusion that the modifier loci for cap color work in a brown population are also the determinants of cap whiteness in a white population.

In conclusion, in this study major QTLs for bruising sensitivity and cap color were assigned to chromosomes of button mushrooms and will thus contributes to our understanding of the genetic base for these traits. However, the very low recombination frequency found in the bisporic varieties used in this paper makes it difficult to assign QTL to chromosomal regions and thus finding candidate genes involved. It also hampers the introduction of the bruising insensitive in commercial lines since linkage drag will impede the restoration of the quality of a commercial receptor variety. This shows the need to introduce a "normal" meiotic recombination found in the *burnettii* variety into breeding stock.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fgb.2015.04.003.

References

Burton, K.S. et al., 1993. Purification and characterization of a serine proteinase from senescent sporophores of the commercial mushroom *Agaricus bisporus*. J. Gen. Microbiol. 139, 1379–1386.

Callac, P. et al., 1993. Morphological, genetic, and interfertility analyses reveal a novel, tetrasporic variety of *Agaricus bisporus* from the Sonoran Desert of California. Mycologia 85, 835–851.

Callac, P. et al., 1998. Evidence for PPC1, a determinant of the pilei-pellis color of *Agaricus bisporus* fruitbodies. Fungal Genet. Biol. 23, 181–188.

Chang, S.-T., Wasser, S.P., 2012. The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. Int. J. Med. Mushrooms 14, 95–134.

Foulongne-Oriol, M. et al., 2010. An expanded genetic linkage map of an intervarietal *Agaricus bisporus* var. *bisporus* × *A. bisporus* var. *burnettii* hybrid based on AFLP, SSR and CAPS markers sheds light on the recombination behaviour of the species. Fungal Genet. Biol. 47, 226–236.

Foulongne-Oriol, M. et al., 2011. Quantitative genetics to dissect the fungal-fungal interaction between *Lecanicillium verticillium* and the white button mushroom *Agaricus bisporus*. Fungal Biol. 115, 421–431.

Foulongne-Oriol, M. et al., 2012. Quantitative trait locus mapping of yield-related components and oligogenic control of the cap color of the button mushroom, *Agaricus bisporus*. Appl. Environ. Microbiol. 78, 2422–2434.

Fritsche, G.G.J.P.G.P.T.V.H.R., 1981. Eigenschappen van de nieuwe rassen Horst U1 en Horst U3. De Champignoncultuur. 25 (8), 345–353.

Gao, W. et al., 2013. Genetic variation and combining ability analysis of bruising sensitivity in *Agaricus bisporus*. PLoS ONE 8, e76826.

Gerritsen, Y.A.M. et al., 1994. The low-isoelectric point tyrosinase of *Agaricus bisporus* may be a glycoprotein. Phytochemistry 35, 573–577.

Jolivet, S. et al., 1998. *Agaricus bisporus* browning: a review. Mycol. Res. 102, 1459–1483.

Kerrigan, R.W. et al., 1993. Meiotic behavior and linkage relationships in the secondarily homothallic fungus *Agaricus bisporus*. Genetics 133, 225–236.

Larraya, L.M. et al., 2002. Quantitative trait loci controlling vegetative growth rate in the edible basidiomycete *Pleurotus ostreatus*. Appl. Environ. Microbiol. 68, 1109–1114.

Mauracher, S.G. et al., 2014a. Crystallization and preliminary X-ray crystallographic analysis of latent isoform PPO4 mushroom (*Agaricus bisporus*) tyrosinase. Acta Crystallogr. F: Struct. Biol. Commun. 70, 263–266.

Mauracher, S.G. et al., 2014b. Latent and active abPPO4 mushroom tyrosinase cocrystallized with hexatungstotellurate(VI) in a single crystal. Acta Crystallogr. D: Biol. Crystallogr. 70, 2301–2315.

Mauracher, S.G. et al., 2014c. High level protein-purification allows the unambiguous polypeptide determination of latent isoform PPO4 of mushroom tyrosinase. Phytochemistry 99, 14–25.

Miller, R.E. et al., 1974. Inheritance of sporophore color and "wild" morphology in *Agaricus bisporus*. Mushroom Sci. 9, 39–45.

Moquet, F. et al., 1999. A quantitative trait locus of *Agaricus bisporus* resistance to *Pseudomonas tolaasii* is closely linked to natural cap color. Fungal Genet. Biol. 28, 34–42.

Morin, E. et al., 2012. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. Proc Natl. Acad. Sci. USA 109, 17501–17506.

Raper, C.A., Raper, J.R., 1972. Genetic analysis of the life cycle of *Agaricus bisporus*. Mycologia 64, 1088–1117.

Soler-Rivas, C. et al., 1997. Activation of tyrosinase in *Agaricus bisporus* strains following infection by *Pseudomonas tolaasii* or treatment with a tolaasin-containing preparation. Mycol. Res. 101, 375–382.

Sonnenberg, A.S. et al., 1999. Abr1, a transposon-like element in the genome of the cultivated mushroom *Agaricus bisporus* (Lange) Imbach. Appl. Environ. Microbiol. 65, 3347–3353.

Sonnenberg, A.S.M. et al., 2005. Breeding mushroom: state of the art. Acta Edulis Fungi (Suppl.) 12, 163–173.

Sonnenberg, A.S.M. et al., 2011. Breeding and strain protection in the button mushroom *Agaricus bisporus*. In: Proceedings of the 7th International Conference of the World Society for Mushroom Biology and Mushroom Products, vol. 1, pp. 7–15. <https://colloque4.inra.fr/icmbmp7/Download-proceedings>.

Stoop, J.M.H., Mooibroek, H., 1999. Advances in genetic analysis and biotechnology of the cultivated button mushroom, *Agaricus bisporus*. Appl. Microbiol. Biotechnol. 52, 474–483.

Straatsma, G. et al., 2007. Systeeminnovatie in teelt en voeding van champignons. Plant Research International, Onderzoeksgroep Paddenstoelen, Wageningen, pp. 27.

Van Leeuwen, J., Wichers, H.J., 1999. Tyrosinase activity and isoform composition in separate tissues during development of *Agaricus bisporus* fruit bodies. Mycol. Res. 103, 413–418.

Weijn, A., 2013. Unravelling the Bruising Discoloration of *Agaricus bisporus*, the Button Mushroom. PhD Dissertation, Wageningen. <http://library.wur.nl/WebQuery/wda/2030852>.

Weijn, A. et al., 2012. A new method to apply and quantify bruising sensitivity of button mushrooms. LWT – Food Sci. Technol. 47, 308–314.

Weijn, A. et al., 2013a. Melanin biosynthesis pathway in *Agaricus bisporus* mushrooms. Fungal Genet. Biol. 55, 42–53.

Weijn, A. et al., 2013b. Main phenolic compounds of the melanin biosynthesis pathway in bruising-tolerant and bruising-sensitive button mushroom (*Agaricus bisporus*) strains. J. Agric. Food Chem. 61, 8224–8231.

Xu, J. et al., 1993. Localization of the mating type gene in *Agaricus bisporus*. Appl. Environ. Microbiol. 59, 3044–3049.