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Blue Light Signaling Inactivates the Mating Type Genes-Mediated Repression of Asexual Spore Production in the Higher Basidiomycete *Coprinopsis cinerea*

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Abstract: Monokaryotic mycelia of several wild-type strains of the homobasidiomycete *Coprinopsis cinerea* form abundant numbers of oidia both in the light and dark due to the regulation of oidia production by the *A* and *B* mating type genes. Nevertheless, little is known about whether and how the mating type loci and light signal regulate the oidiation in *C. cinerea*. Herein, the experimental results demonstrated that the self-compatible homokaryon AmutBmut strain, the mycelia whose nuclei carry mutations in both the *A* and *B* loci, can produce only a few oidia in the dark, whereas the formation of numerous numbers of oidia is induced by the light. The semi-compatible homokaryon AmutB, but not ABmut, has the production and behavior of oidia formation similar to those of AmutBmut. These findings indicated that in AmutBmut strain the mutation at the *A* locus results in repression of oidiation in the dark and the blue light alleviates this effect, whereas the mutated *B* genes function has no effects. Since, the oidia production relies on both *A* and light signal, it is possible that *A* locus might be linked to the blue light receptor genes. The present results demonstrated for the first time that the secondary hyphal knot formation (*skn1*), fruiting body maturation (*mat*) and basidiospore formation (*bad*) genes which are essential in the *C. cinerea* fruiting pathway are not involved in the regulation of asexual sporulation. In addition, the positive light effect on oidiation could also occur in *C. cinerea* dikaryons.

Key words: Oidiation, monokaryon, dikaryon, mating types, light, *Coprinopsis cinerea*

INTRODUCTION

Coprinopsis cinerea is a heterothallic basidiomycete i.e., it has two types of mycelia in its life cycle, namely the sterile primary mycelium (monokaryon) and the fertile secondary mycelium (dikaryon). The monokaryon has simple septa and forms asexual spores (oidia) constitutively in abundant numbers on the specialized aerial hyphae (oidiophores) (Kües, 2000; Kües *et al*., 1998, 2002). The dikaryon is generated by mating of the two compatible monokaryons. Dikaryon contains two genetically distinct nuclei in a hyphal cell. The dikaryotic mycelium has clamp cells at each septum and develops under appropriate conditions into the fruiting body (Moore, 1984) in which karyogamy and meiosis occur on the specialized structure (basidium) and eventually the meiotic basidiospores are produced (Casselton, 1978). It has long been assumed that the dikaryons either produce no asexual spores (Brodie, 1931; Swamy *et al*., 1984) or produce at a low level (Swamy *et al*., 1984).

In higher basidiomycetes, monokaryon compatibility and mating is regulated by two multiallelic independent mating type loci, namely *A* and *B* (Casselton and Kües, 1994; Casselton and Olesnicky, 1998). Molecular analysis of the *A* and *B* mating type genes in *C. cinerea* have revealed that *A* genes code for two distinct types of homeodomain transcription factors (HD1 and HD2) that, to be functional, the HD1 and HD2 proteins from one nucleus must interact with HD2 and HD1 proteins of another compatible nucleus respectively to form heterodimers (Pardo *et al*., 1996; Kües, 2000; Kamada, 2002; Srivilai *et al*., 2005), whereas *B* genes code for pheromones and their receptors (Casselton and Olesnicky, 1998; Riquelme *et al*., 2005; Casselton and Challen, 2006).

Homokaryon AmutBmut, the mycelia whose nuclei carry mutations in both the *A* and *B* mating type loci, is a self-compatible strain that can develop into the fruiting body without prior mating with a compatible strain (Swamy *et al*., 1984; Liu *et al*., 2006). Recently, the

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AmutBmut *C. cinerea* co-isogenic monokaryotic strains with different mating type specificities PS001-1 (*A42 B42*) and PS002-1 (*A3 B1*) have been constructed from the monokaryons JV6 and 218, respectively, by the repetitive backcrossing for 6 generations with a fixed parental self-compatible homokaryon AmutBmut strain and were employed to analyze the vital functions of several unknown genes which may be silent in the genome (Liu *et al*., 2006; Srivilai *et al*., 2005). In this study, *C. cinerea* co-isogenic strain PS001-1 was crossed with the homokaryon AmutBmut strain (*A43mut B43mut*) and the A43mutB42 and A42B43mut progenies were isolated to further determine whether and how the mating type genes affect the mycelial growth and development and also the asexual sporulation.

Blue light is known to be one of the most important environmental signals for various organisms, including fungi, for regulating their growth, developmental and physiological processes. It is appeared that blue light signaling induces the proper fruiting body development in *C. cinerea* (Kües *et al*., 1998). Blue light receptors have been found in several fungi, including the basidiomycete *C. cinerea* and the ascomycete *Neurospora crassa* (Sano *et al*., 2007). A blue light photoreceptor of the *N. crassa*, White Collar-1 (WC-1), has been cloned in *C. cinerea* from a mutant which is defective in the light regulation during fruiting body development, suggesting that WC-1 is an essential component of all known blue light responses (Sano *et al*., 2007). Most recently, a blue light receptor gene, *Dst1*, was investigated in *C. cinerea* (Terashima *et al*., 2005; Sano *et al*., 2007). *Dst1* gene encodes a deduced protein which consists of 1,175 amino acid residues and contains two sensory domains superfamily PAS (Per-Arnt-Sim), i.e., PAS-A and PAS-B, a coiled-coil structure and a putative, glutamine-rich, transcriptional Activation Domain (AD). One of the PAS domains exhibits significant similarity to the LOV (PAS-A) domains of known blue light receptors, suggesting that Dst1 is a blue light receptor of *C. cinerea* (Terashima *et al*., 2005; Sano *et al*., 2007).

Amongst higher basidiomycete, the homobasidiomycete *C. cinerea* is a unique organism with a dramatic morphological differentiation from vegetative mycelia into fertile fruiting bodies in which abundant numbers of basidiospores are formed. Deepening the understanding of the growth and developmental processes underlying fruiting initiation and development and asexual spore production in this mushroom model is expected to help in the future the world mushroom cultivation of any other basidiomycetes concerning the potential agronomic, economic and environmental benefits. In the earlier studies, there has been previously demonstrated for the first time the molecular mechanism and essential genes function in fruiting body development in *C. cinerea* and noted that the coordinated regulations of both the *A* and *B* mating type loci are essential for fruiting initiation and sexual development in *C. cinerea* (Srivilai *et al*., 2005; Liu *et al*., 2006). However, till date very little has been known about whether and how the mating type loci and blue light signal regulate the asexual sporulation in *C. cinerea*. To address this issue, the present study is an attempt to evaluate the effects of the mating type genes on asexual spore production under both light and dark conditions by utilizing the homokaryon AmutBmut strain carrying mutations in both two mating type loci, the semi-compatible A43mutB42 and A42B43mut strains having either a mutation in *A* or *B* locus and the co-isogenic monokaryotic strains PS001-1 and PS002-1 as the experimental models. Furthermore, the *C. cinerea* AmutBmut mutant strain 6-031 possessing the mutated *skn1*, *mat* and *bad* genes was employed to additionally determine whether the effects of these crucial genes involved in the fruiting development pathway on the asexual spore production both in the light and in the dark.

MATERIALS AND METHODS

This study was carried out from 2007 to 2008 at Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham, Thailand.

***C. cinerea* strains and growth conditions:** *C. cinerea* strains used in this study were the monokaryons JV6 (*A42 B42*), AT8 (*A43 B43*, *trp-3*, *ade-8*), 6-031 (*A43mut B43mut*, *pab-*, *skn-*, *mat-*, *bad-*), PS001 (*A42 B42*), PS001-1 (*A42 B42*; AmutBmut co-isogenic monokaryon), PS002 (*A3 B1*), PS002-1 (*A3 B1*; AmutBmut co-isogenic monokaryon), 218 (*A3 B1*, *trp-1.1*,*1.6*, *bad*) and Okayama 7 (*A43 B43*), homokaryon AmutBmut (*A43mut B43mut*, *pab-*), semi-compatible homokaryons A43mutB42 (*A43mut B42*, *pab-*) and A42B43mut (*A42 B43mut*), which were derived from a mushroom (PS001-1 × AmutBmut). Identification of the *A43mut B42* and *A42 B43mut* genotypes was carried by crossing with the suitable monokaryotic tester strains and selected by growing on selective medium supplemented with parabenzoic acid as previously described (Liu *et al*., 2006). Cultures were standardly grown at 37°C in 9 cm diameter Petri dishes containing YMG/T (Yeast extract-Malt extract-Glucose, supplemented with Tryptophan) complete medium (Granado *et al*., 1997). Four pieces of small squares of mycelia (0.5×0.5 cm) were placed in the middle of YMG/T plates and the cultures were grown by incubation at 37°C

in light-proof, ventilated boxes until the plates were fully covered with mycelia. A half of total cultures were transferred from the dark to light condition (light intensity of 20-25 μE m^{-2} sec^{-1} provided by the standard white fluorescence tubes (Toshiba, L40/25; main emission spectrum 295-780 nm). The cultures were further incubated at 37°C for 5 consecutive days, whilst another subset of cultures was further kept in the dark.

Harvesting and counting of asexual spores: The standard oidiation test was conducted as described previously by Kertesz-Chaloupková *et al.* (1998). Briefly, oidia were harvested in 10 mL water by scraping the aerial mycelium from the agar surface of the culture plate with a blunt scalpel. The oidia were separated from mycelial debris by filtration through a glass funnel containing glass wool. The number of oidia per culture was determined by counting in a Thoma chamber and the mean values were calculated from four individual samples.

Statistical analysis: Data were presented as Mean±SD. The significant differences among all of the treatment groups were analyzed by one-way ANOVA with the Tukey post-hoc test followed by the Student's t-test (PrismTM; GraphPad, San Diego, CA, USA). $p<0.05$ were considered significant.

RESULTS

No significant effect of blue light signaling on asexual sporulation in *C. cinerea* monokaryons: It has long been believed that light does not play a role in asexual basidiospore formation in *C. cinerea* (Brodie, 1931). To confirm this assumption, several *C. cinerea* monokaryons i.e., JV6 (*A42 B42*), AT8 (*A43 B43*, *trp-3*, *ade-8*), 6-031 (*A43mut B43mut*, *pab-*, *skn-*, *mat-*, *bad-*), PS001 (*A42 B42*), PS001-1 (*A42 B42*; AmutBmut co-isogenic monokaryon), PS002 (*A3 B1*), PS002-1 (*A3 B1*; AmutBmut co-isogenic monokaryon), 218 (*A3 B1*, *trp1.1,1.6*, *bad*) and Okayama 7 (*A43 B43*) strains which carry the different genetic backgrounds and various *A* and *B* mating type specificities were tested for their ability to produce vegetative basidiospores (oidia) on the YMG/T complete medium. The fully grown cultures were kept separately under the dark and light conditions at 37°C for 5 consecutive days. At day 5, the oidia were harvested from each culture and then counted. The results showed that the numbers of oidia produced per culture were varied among monokaryons with different genotypes, but light did not exert any significant effect on oidia production in these monokaryotic strains (Fig. 1).

Light induces oidia production in all AmutBmut homokaryons and its fruiting mutant, 6-031: The homokaryon AmutBmut and 6-031 strains with the constitutive Aon and Bon phenotypes were tested for their ability to produce oidia on the YMG/T plates. The fully grown cultures were kept at 37°C in the dark or in the light for 5 consecutive days. The results indicated that in contrast to monokaryons the homokaryon AmutBmut produced a very low number of oidia in the dark, with the average values of 5.0×10^7 oidia per culture (Fig. 1, 2).

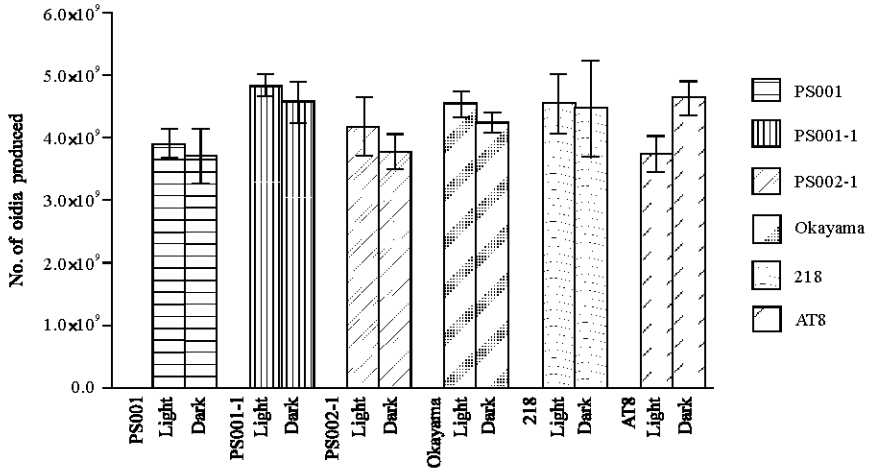

Fig. 1: Comparison of oidia production in various *C. cinerea* monokaryotic strains. The fully-grown cultures were incubated separately under light or dark condition on YMG/T medium at 37°C for 5 consecutive days. The oidia were then harvested and counted. Data represent Means±SD, n = 4-6/group from at least two independent experiments. No significant effect of light signaling on oidiation of these strains was observed

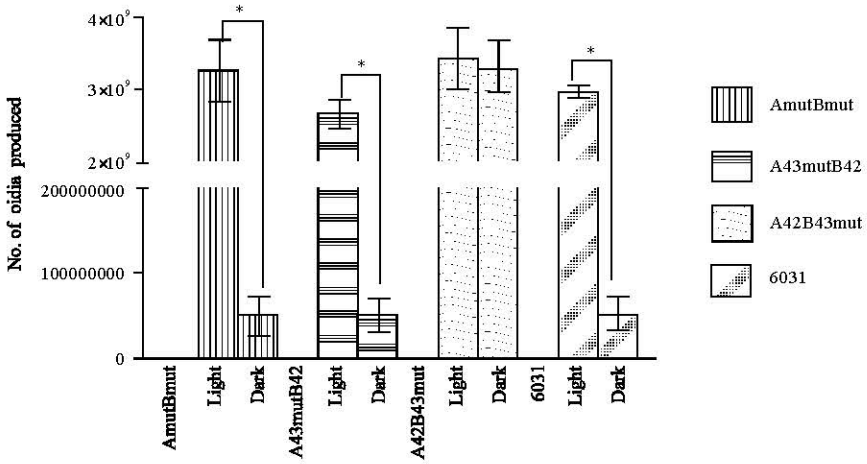

Fig. 2: Comparison of oidia production of the homonokaryon AmutBmut, A43mutB42, A42B43mut and 6031 strains. The fully-grown cultures were incubated separately in the light or in the dark on YMG/T medium at 37°C for 5 consecutive days. The oidia were then harvested and counted. Each bar represents Mean±SD, n = 4-6/group from at least two independent experiments. *$p<0.05$ means the significant difference found in each treatment when compared between under light and dark conditions

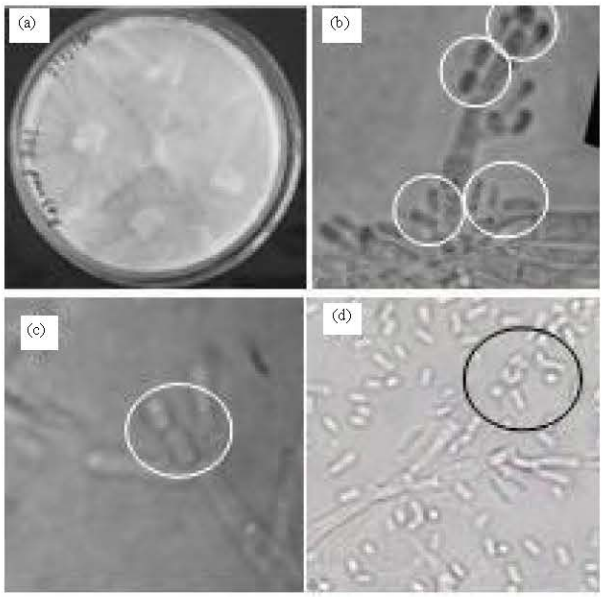

Fig. 3: The fully grown culture of A43mutB42 homokaryon on the YMG/T complete medium at day 5 (a); Upon light exposure, the semi-compatible A43mutB42 homokaryons produced high amount of oidia (encircled) which formed on the aerial hyphae (100x) (b); In the dark, less number of oidia (encircled) of A43mutB42 strain were formed on the aerial hyphae (100x) (c) and Numerous oidia (encircled) of the AmutBmut co-isogenic monokaryons PS001-1 in the dark (40x) (d)

However, when fully grown cultures were exposed to the light the numbers of oidia produced per culture were dramatically increased, with the average values of 3.3×10^9 oidia per culture and reached to the values comparable to those in monokaryons (Fig. 1, 2). Similarly, the fruiting mutants of AmutBmut, the UV-mutant 6-031 which having also the mutations at three different genes importantly involved in fruiting body development in *C. cinerea*, i.e., secondary hyphal knot formation (*skn1*), fruiting body maturation (*mat*) and basidiospore formation (*bad*) genes, produced a very low number of oidia in the dark, with the average values of 5.3×10^7, but oidia formation was

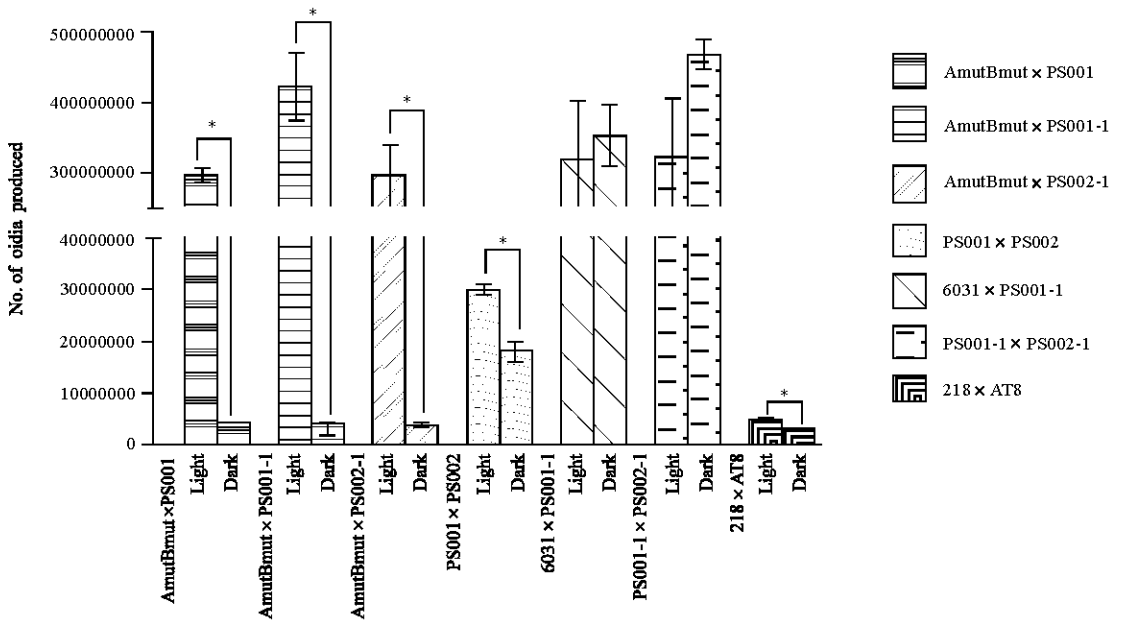

Fig. 4: Comparison of oidia production of *C. cinerea* dikaryons having different genetic backgrounds. The fully-grown cultures were incubated separately under light or dark condition on YMG/T medium at 37°C for 5 consecutive days. The oidia were then harvested and counted. Values represent Means±SD, n = 4-6/group from at least two independent experiments. *p<0.05 means the significant difference found in each treatment when compared between under the light and dark conditions

induced up to the average numbers of 2.9×10^9 oidia per culture upon blue light exposure (Fig. 2).

***Amut* function causes repression of oidiation in the dark, whereas *Bmut* function is not effective in AmutBmut homokaryons:** Since blue light had a very potent effect on induction of asexual sporulation in all AmutBmut homokaryons and its fruiting UV-mutant 6031 but the inductive effect of blue light on oidiation was not observed in all investigated monokaryons, it was therefore hypothesized that one or both mating type loci may be responsible for regulation of oidiation. In order to identify which mating type gene functions in regulation of oidia production, the AmutBmut co-isogenic monokaryon PS001-1 (*A42 B42*) was crossed with homokaryon AmutBmut strain (*A43mut B43mut*) and the progenies carrying the *A43mut B42* (an *Amut* and *B* wild-type genotype) and *A42 B43mut* (an *A* wild-type and *Bmut* genotype) genotypes were randomly isolated to test for their ability to produce oidia on the YMG/T medium. The results indicated that the numbers of oidia produced under dark condition of all investigated A43mutB42 homokaryons with the mutation at the *A* mating locus were very low and reached the values comparable to those found in the AmutBmut homokaryons (Fig. 2, 3c). In contrast, in the light these A43mutB42 homokaryons produced markedly large numbers of oidia per culture which reached values comparable to those observed in monokaryons (Fig. 2, 3b) as well as the AmutBmut homokaryons (Fig. 2). On the other hand, the homokaryon A42 B43mut, the mycelia whose nuclei carry mutation at the *B* mating locus, produced a high number of oidia in the light, with the average amounts of about 3.4×10^9 oidia per culture, however the light signaling did not significantly exert any inductive effect on oidiation in this strain, i.e., the numbers of oidia produced per culture of the A42B43mut monokaryons were not significantly different between under light and dark conditions (Fig. 2).

Oidia production in dikaryons is regulated by mating type genes: Herein, this study demonstrated that in the light the AmutBmut strains can produce abundant numbers of oidia typical of monokaryons. It is appeared that the AmutBmut homokaryons show growth characteristics of dikaryons, i.e., formation of clamp cells and differentiation into fruiting bodies. Hence, it is possible that like homokaryons, dikaryons would produce low number of oidia in the dark and that the light-induced asexual sporulation would still exist in dikaryons. To elucidate this assumption, mating experiments were conducted between any two compatible monokaryons

(PS001 × PS002; PS001-1 × PS002-1; 218 × AT8; 6-031 × PS001-1), or between a compatible monokaryon and the homokaryon AmutBmut (AmutBmut × PS001; AmutBmut × PS001-1; AmutBmut × PS002-1). The results showed that all investigated dikaryons formed by the combinations of homokaryon AmutBmut and the compatible monokaryons produced a very low number of oidia in the dark comparable to those in AmutBmut homokaryons. However, when fully grown cultures were exposed to the light the numbers of oidia produced per culture were dramatically increased by factor of ~100 fold and reached to the values comparable to those observed in AmutBmut homokaryons (Fig. 4, 2). However, in the light the numbers of oidia produced in dikaryons formed by the homokaryon AmutBmut and the monokaryons were at about 13 fold less than those found in the corresponding parental monokaryotic strains. In contrast, the oidia produced from all investigated dikaryons formed by two compatible monokaryons varied considerably in number from dikaryon to dikaryon (Fig. 4). Nevertheless, the numbers of oidia formed in these dikaryons in the light were greatly lower than those observed in the corresponding parental monokaryotic strains (Fig. 4, 2). The dikaryons formed by the crosses of PS001 × PS002 and 218 × AT8 have significantly lower numbers of oidia formed in the dark than in the light in the similar pattern but to greatly lower degree of change found in the dikaryons formed by the homokaryon AmutBmut and the compatible monokaryons (Fig. 4).

DISCUSSION

The objective of the present study was the investigation of the putative effects of the mating type genes along with the blue light signaling on asexual spore production in the heterothallic basidiomycete *C. cinerea* monokaryons as well as dikaryons. As the experimental models the self-fertile homokaryon AmutBmut strain carrying mutations in both the *A* and *B* mating type loci, the semi-compatible A43mutB42 and A42B43mut strains having either a mutation in *A* or *B* mating type locus were employed. Furthermore, to evaluate the possible effects of other crucial genes than the mating type genes on regulation of the oidia production, the *C. cinerea* AmutBmut UV-mutant strain 6-031 possessing the mutated *skn1*, *mat* and *bad* genes in the fruiting pathway was used to determine such effects under both light and dark conditions.

According to this study, it was found that the numbers of oidia produced in all of the tested monokaryotic strains are at relatively high levels both in the light and in the dark. These current results confirm and extend those previously reported by Brodie (1931) and Kertesz-Chaloupková *et al.* (1998), who demonstrated that the monokaryotic mycelia produced abundant numbers of oidia and light signaling does not play a role on oidiation in *C. cinerea* monokaryons. These results could be explained through the investigations that monokaryons do not possess the compatible HD1-HD2 heterodimer from *A* mating type genes, an active A protein combination, to acts as a repressor of oidiation in the dark, but not in the light (Kertesz-Chaloupkova *et al.*, 1998; Kües *et al.*, 2002). According to this current study, the results also indicated that a 5-day treatment regimen is already sufficient to unravel the effects of either the mating type genes, or blue light signaling on the production of oidia in this heterothallic fungal species.

To unravel whether the effects of either *A*, or *B* mating type genes along with the blue light illumination on the regulation of oidia production, the AmutBmut, A43mutB42 and A42B43mut homokaryons, which have been currently accepted as the reliable experimental models (Srivilai *et al.*, 2005; Liu *et al.*, 2006), were employed in this study. Consistent with earlier studies, the present results show that mutations in both the *A* and *B mating* type loci of the *C. cinerea* AmutBmut strain resulted in a very low number of oidia produced per culture in the dark, while upon blue light exposure AmutBmut homokaryons produced relatively high number of oidia as those found in the wild-type monokaryons (Polak *et al.*, 1997; Kertesz-Chaloupkova *et al.*, 1998). Likewise, the recent data from this study demonstrated the first evidence that in the fruiting mutant of AmutBmut, the UV-mutant 6-031, which shows the defects in the transition from the primary- to secondary hyphal knot, the fruiting body maturation and the formation of the black-coloured basidiospores due to the mutations at *skn1*, *mat* and *bad* genes, respectively (Srivilai *et al.*, 2005; Liu *et al.*, 2006), the oidiation was also repressed in the dark and dramatically increased in the light. It is therefore concluded that either the *A* or *B* mating type genes, but not the *skn1*, *mat* and *bad* genes, could be responsible for repression of oidia production in the dark and the blue light displays the inductive effects. On the other hand, however, all investigated A43mutB42 homokaryons with the mutation in the *A* mating genes formed very low numbers of oidia in the dark, but in the light these A43mutB42 homokaryons produced significantly high numbers of oidia per culture which reached values comparable to those observed in monokaryons and the AmutBmut homokaryons. In contrast, the A42B43mut, the mycelia whose nuclei carry mutation in the *B* mating locus, produced relatively high numbers of oidia both in

the dark and in the light. As determined from the genotypes along with the phenotypes, it is appeared that the activated *Amut*-, but not *Bmut*-function causes repression in oidiation and thus induction of fruiting body development as previously demonstrated by Kües *et al.* (2002). Altogether, in consistent to the earlier data reported by Kertesz-Chaloupková *et al.* (1998) the present findings clearly indicate the involvement of the blue light signaling in regulation of asexual sporulation by overriding the *A*-mediated repression of oidia production, whereas the *B* function cause reduction in the positive light effect on *A*-mediated repression of oidiation but this effect is not active in the AmutBmut homokaryons. Therefore, it may be possible that the blue light receptor genes might be linked to the *A* mating type genes. Consequently, the mutation in *A* genes would also affect the functioning of blue light receptor genes as indicated by occurrence of very low numbers of oidia produced in the dark in A43mutB42 when compared with those of A42B43mut. Thus, the relatively high numbers of oidia production in A43mutB42 are strongly dependent on blue light exposure as previously reported by Kertesz-Chaloupková *et al.* (1998).

To the best of our knowledge, the present results demonstrated for the first time that the *skn1*, *mat* and *bad* or *spo* genes which are essential in the *C. cinerea* fruiting body development pathway are not involved in the regulation of asexual sporulation under both light and dark conditions as indicated by the occurrence of a very low level of numbers of oidia produced in the dark and the dramatically increased oidia production via light induction in the similar pattern found in those of AmutBmut and AmutB42 homokaryons. These data therefore indicated that there are no correlated signaling pathways of fruiting body development and oidiation in *C. cinerea*. However, it was reported that about one-third of mutants defective in fruit body initiation are also defective in oidiation (Kües *et al.*, 2001). Hence, the asexual differentiation is dependent upon only the genes involved in fruiting initiation, not those involved in fruiting body development. In addition, these data implied that several of the other AmutBmut mutant strains that are blocked at different stages in fruiting pathway due to the mutation of genes affecting fruiting development in *C. cinerea* (Srivilai *et al.*, 2005; Liu *et al.*, 2006) can be used to develop a reliable mushroom model for assessing putative effects of those mutated genes in regulation of oidia production rather than the defined mating type genes.

It has been previously reported that dikaryotic mycelia produced no or very low number of asexual spores due to the presence of the active heterodimers from *A* mating type genes of different parental origin which consequently causes the suppression of asexual spore production on the aerial mycelium of the dikaryon under dark condition (Swamy *et al.*, 1984; Polak *et al.*, 1997; Kües *et al.*, 2002). Consistent with earlier studies, the current study found that the AmutBmut strain can produce in a light-dependent manner abundant numbers of oidia typical of monokaryons (Polak *et al.*, 1997; Kertesz-Chaloupkova *et al.*, 1998). However, it has been revealed that the AmutBmut homokaryons show growth characteristics typical of dikaryons, i.e., formation of clamp cells at hyphal septa and differentiation into fruiting bodies without prior mating to another strain (Swamy *et al.*, 1984). Therefore, it may be possible that like homokaryons, dikaryons would produce a very low number of oidia in the dark and that the light signaling would induce constitutively asexual sporulation in dikaryons. The experimental results demonstrated that in the dark all investigated dikaryons formed by the combinations of homokaryon AmutBmut and the compatible monokaryons produced some quantities of oidia but at the very low levels comparable to those found in the AmutBmut homokaryons and that the repression of oidia production was significantly alleviated upon blue light exposure although the numbers of oidia produced were relatively less than those found in the corresponding parental monokaryotic strains. These data are in line with the observations that in *C. cinerea* dikaryons the compatible products of the *B* locus could reduce the outcome of light-induced effect on *A*-mediated repression of oidiation and the coordinated regulation of oidia production by compatible *A* and *B* genes results in less number of oidia production in light than those found in monokaryons. In addition, these results suggested that the positive light effect on oidiation could also occur in *C. cinerea* dikaryons as well as homokaryons as previously described by Kertesz-Chaloupkova *et al.* (1998). Interestingly, the results showed that the numbers of oidia produced from all investigated dikaryons formed by two compatible monokaryons were varied considerably from dikaryon to dikaryon. This phenomenon is most probably due to the large heterogenous genetic backgrounds of their respective parental monokaryotic strains. It has been known that each *C. cinerea* strain gives the morphological variants of oidiophores (Polak *et al.*, 1997; Kertesz-Chaloupková *et al.*, 1998; Fischer and Kües, 2006) which may result in the considerable variation in the number of oidia produced across the dikaryotic strains. However, the numbers of oidia produced per culture of two investigated dikaryons, i.e., 6-031 × PS001-1 and PS001-1 × PS0002-1 were not significantly different when compared between under light

and dark conditions, unlike those of the other two tested dikaryons 218 × AT8 and PS001 × PS002. Nevertheless, in the light the numbers of oidia formed in these dikaryons with different *A* and *B* mating type specificities were greatly lower than those in the corresponding parental strains. This discrepancy cannot be explained clearly at present; however, it has been demonstrated previously that depending upon the genetic backgrounds of the parental strains, in *C. cinerea* dikaryons the regulation of oidia production may be controlled independently of light (Kertesz-Chaloupková *et al.*, 1998). In addition, these findings indicated that the compatible products of *B* mating type locus may modulate the oidia production in these dikaryotic mycelia by overriding the effect of light on releasing the *A*-mediated repression of oidiation as already mentioned.

CONCLUSION

Taken together, these results strongly revealed that oidia production in all investigated *C. cinerea* monokaryons is not significant different between in the light and dark, whilst the numbers of oidia produced by several dikaryons with different mating type specificities are varied considerably depending upon their genetic backgrounds. The asexual sporulation in homokaryon AmutBmut strain was repressed in the dark, but the production of oidia could be effectively induced by light to the numbers comparable to those observed in monokaryons. A certain amount of light-induced oidiation was also observed in all AmutB42, but not in A42Bmut homokaryons. In dikaryons, the light induction overrides the effect of *A*-mediated repression of oidiation over a range of strain with defect in *A* mating type genes, whilst the *B* mating type genes counteract the effects of light-mediated release of repression of oidiation by *A* mating type genes, resulting in production of less oidia and the *Bmut* function in the AmutBmut homokaryons is appeared not to affect the asexual sporulation. Since the oidia production relies on both the *A* mating type genes and blue light signal, it is therefore postulated that the blue light receptor genes might be linked to the *A* mating type genes and this warrants further studies. In addition, this current study demonstrated the first evidence that the *skn1*, *mat* and *bad* genes affecting fruiting body development in *C. cinerea* do not affect oidiation. Further mutants with defects in any of other genes involved in the development of *C. cinerea* fruiting body, i.e., primary hyphal knot (*pkn*), primordium maturation (*prm*), stipe elongation (*eln*) and cap expansion (*exp*) are in the line of ongoing investigations.

In summary, the present findings that the *C. cinerea* dikaryons, as well as homokaryons, produce abundant numbers of oidia upon blue light induction and furthermore the blue light-induced oidiation does not under the control of genes affecting the development of fruiting body provide the better understanding and a new knowledge of the regulation of asexual spore production in this mushroom model which is expected to help in the future the world mushroom cultivation of any other basidiomycetes concerning the potential agronomic, economic and environmental benefits.

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