

Short Communication

Inheritance of photo-control of conidial development in the fungus *Bipolaris oryzae*

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The inheritance of light dependence for conidial development in *Bipolaris oryzae* was analyzed using single-ascospore isolates. When a photo-induced strain was crossed with another photo-induced strain, only photo-induced progeny were produced. When a photo-induced strain was crossed with a non-photo-induced (I) strain, photo-induced and non-photo-induced (I) progeny were produced in a ratio of 1 : 1. However, when a photo-induced strain was crossed with a non-photo-induced (II) strain, a non-photo-induced (I) progeny were formed in addition to parental types. It was suggested that genes for photo-control of conidiophore induction and genes for photo-control of conidiophore maturation are located on the same chromosome.

Key Words—*Bipolaris oryzae*; inheritance; mating; myochrome system; photo-control of conidial development.

It has been reported that conidial development in certain fungi imperfecti such as *Alternaria* species, *Botrytis cinerea* Pers.: Fr., and *Bipolaris oryzae* (Breda de Haan) Shoem. is controlled by the antagonistic action of blue/UV-A and near-UV radiation mediated through the myochrome system (Honda et al., 1968; Kumagai, 1978, 1988). We previously reported that there are three phenotypes of *B. oryzae*, photo-induced, non-photo-induced (I), and non-photo-induced (II) sporulators, based on the light requirements for conidiophore induction and conidiophore maturation (Kihara and Kumagai, 1994). Conidiophore formation in the photo-induced sporulator of *B. oryzae* is induced by near-UV radiation, and conidia develop in the subsequent darkness. However, the inductive effect of near-UV radiation can be nullified by blue radiation applied immediately after inductive near-UV radiation. When conidiophores are exposed to blue radiation at a certain conidiophore maturation stage, they dedifferentiate into sterile conidiophores, and conidia do not form. However, the inhibitory effect of blue radiation on conidial development can be nullified by near-UV radiation. In a non-photo-induced (I) sporulator of *B. oryzae*, although conidiophore formation can be induced irrespective of light or dark conditions, conidial development following conidiophore formation is photo-controlled by the antagonistic action of blue/UV-A and near-UV radiation mediated through the myochrome system (Yamamura et al., 1978). On the other hand, in the non-photo-induced (II) sporulator, neither induction of conidiophore induction nor conidial development following conidiophore formation was affected by light conditions. Furthermore, we found that photo-induced sporulator of *B. oryzae* constituted the majority of isolates collected from rice plant cultivated in various paddy

fields, while only a few non-photo-induced isolates existed (Kihara et al., 1997).

Chang (1981) reported that genes for light-dependence for conidiophore induction and genes for photo-inhibition of conidiophore maturation in *Cochliobolus miyabeanus* (Ito & Kuribayashi) Drechsler ex Dastur, the teleomorph of *B. oryzae*, are located on different chromosomes, i.e., they are not linked. In his study, however, the light dependence of conidial development was merely examined under continuous irradiation with white light or under continuous darkness, but neither light requirement nor light quality for conidiophore induction and conidiophore maturation was specifically considered. In this paper, we report the inheritance of light dependence for conidial development in *B. oryzae* using single-ascospore isolates of progeny from crosses between photo-induced, non-photo-induced (I), and non-photo-induced (II) isolates.

Photo-induced, non-photo-induced (I), and non-photo-induced (II) strains of *B. oryzae* (Kihara and Kumagai, 1994) were used as parental strains. Mating between photo-induced and non-photo-induced isolates was undertaken in rice straw on Sachs agar medium according to Ueyama and Tsuda (1975) and Tsuda and Ueyama (1975). The Petri dish cultures were kept in darkness at $25 \pm 1^\circ\text{C}$ for 30 d. We first examined the compatibility between crosses of isolates belonging to three different photo-sporulator phenotypes in the following combinations: 25 photo-induced isolates \times 1 non-photo-induced (I) isolate; 25 photo-induced isolates \times 2 non-photo-induced (II) isolates; 1 non-photo-induced (I) isolate \times 2 non-photo-induced (II) isolates; and 10 photo-induced isolates \times 10 photo-induced isolates. Among the crosses examined, mature pseudothecia with ascospores in

asci were produced in pairings of photo-induced strain F6 and photo-induced strain S17-2, strain F6 and non-photo-induced (I) strain D6, and strain F6 and non-photo-induced (II) strain D9. In other pairings, pseudothecia neither formed nor matured. Ueyama and Tsuda (1976) reported that *C. miyabeanus*, the teleomorph of *B. oryzae*, is heterothallic with the mating type alleles *A* and *a* being required for sexual reproduction, but that the pseudothecia-forming ability can be lost during stock of strains. It is therefore speculated that the failure to produce mature pseudothecia in our experiments might be due to the loss of mating ability in isolates.

Pseudothecia with mature ascospores were crushed, and single ascospores were randomly isolated. Single-ascospore isolates were grown on PDA at $25 \pm 1^\circ\text{C}$ in total darkness for 5 d, under continuous exposure to black light or blue light for 5 d, or under continuous exposure to black light for 4 d followed by darkness or exposure to blue light for 1 d. This procedure was undertaken to judge the classification of isolates into photo-induced, non-photo-induced (I), or non-photo-induced (II) sporulators (Table 1). Black light lamps (FL-BLB, Toshiba, Tokyo, Japan) were used as the source of near-UV radiation, the irradiance being about 540 mW/m^2 . Blue light was obtained from colored fluorescent lamps (20 B-F, Toshiba, Tokyo, Japan), the irradiance being about 370 mW/m^2 . The fluence rates were measured by a compensated vacuum thermopile with a nanovolt meter and an AC amplifier (Japan Spectroscopic, Tokyo, Japan).

When 80 single-ascospore isolates of progeny from a cross between photo-induced strain F6 \times photo-induced strain S17-2 were examined, none of the isolated progeny formed conidiophores or conidia under either total darkness or continuous blue irradiation. When colonies grown in black light for 4 d were transferred to darkness for 1 d, all the isolated progeny developed conidia. However, when blue radiation was applied after the exposure to black light, conidiophores which had formed dedifferentiated into sterile ones, and conidia did not form. Under continuous irradiation with black light, conidiophores which had formed remained conidiophores but conidia did not develop: this effect was due to the balance of the near-UV light component effective for conidiation and the blue light component effective for

suppressing conidiation contained in black light (see Kumagai (1978)). These results indicated that only photo-induced progeny were produced by crossing two photo-induced strains (Table 2).

On the other hand, when 98 single-ascospore isolates of progeny of photo-induced strain F6 \times non-photo-induced (I) strain D6 cross were examined, 48 isolates were found to be photo-induced sporulators and 50 isolates were non-photo-induced (I) sporulators (Table 2). The ratio of these two sporulator types did not deviate significantly from 1 : 1 (χ^2 : 0.04). In non-photo-induced (I) isolates of progeny, conidia developed in total darkness. However, under continuous irradiation with black light, conidiophores which had formed remained conidiophores but conidia did not develop. Hence, conidia developed in the dark following exposure to black light, but conidial development was suppressed by blue radiation following exposure to black light. When 141 single-ascospore isolates of the progeny of photo-induced strain F6 \times non-photo-induced (II) strain D9 cross were examined, the numbers of photo-induced, non-photo-induced (I), and non-photo-induced (II) isolates were 78, 13 and 50, respectively (Table 2). The proportion of photo-induced and non-photo-induced isolates was close to 1 : 1 (χ^2 : 1.60). It should be noted that non-photo-induced (I) isolates appeared in addition to parental types by mating photo-induced with non-photo-induced (II) strains.

Table 2. Segregation of light requirement for conidiation in *B. oryzae*.

Cross	Number of isolates of progeny ^{a)}			χ^2 b)
	PI	NPI-I	NPI-II	
F6 (PI) \times S17-2 (PI)	80	0	0	—
F6 (PI) \times D6 (NPI-I)	48	50	0	0.04
F6 (PI) \times D9 (NPI-II)	78	13	50	1.60

a) PI, photo-induced sporulator; NPI-I, non-photo-induced (I) sporulator; NPI-II, non-photo-induced (II) sporulator.

b) χ^2 values of photo-induced and non-photo-induced progeny type were calculated on the null hypothesis that the segregation ratio is 1 : 1.

Table 1. Conidiation of photo-induced and non-photo-induced sporulators of *B. oryzae* under various light conditions.^{a)}

Sporulator type	Light treatments				
	5 d of darkness	5 d of black light	5 d of blue light	4 d of black light + 1 d of darkness	4 d of black light + 1 d blue light
Photo-induced sporulator	—	— ^{b)}	—	+	—
Non-photo-induced (I) sporulator	+	— ^{b)}	—	+	—
Non-photo-induced (II) sporulator	+	+	+	+	+

a) Experimental fungi were grown under total darkness for 5 d, continuous exposure to black light or blue light for 5 d, or under continuous exposure to black light for 4 d followed by darkness or exposure to blue light for 1 d.

b) Conidiophores remained conidiophores but conidia did not develop.

Suppose that genes for photo-control of conidiophore induction and genes for photocontrol of conidiophore maturation are designated as *A* and *B*, respectively, and that phenotypes of photo-induced, non-photo-induced (I), and non-photo-induced (II) sporulators are designated as *AB*, *aB* and *ab*, respectively. This supposition is consistent with the fact that the proportion of photo-induced and non-photo-induced (I) sporulator isolates of progeny did not deviate significantly from 1 : 1 when photo-induced and non-photo-induced (I) isolates were crossed. On the other hand, when photo-induced and non-photo-induced (II) sporulator isolates were crossed, recombination occurred, i.e., non-photo-induced (I) isolates (*aB*) were produced in addition to the parent types, and the proportion of photo-induced and non-photo-induced (I) plus (II) isolates of progeny was close to 1 : 1. Theoretically, recombinants having genes *Ab* should appear, too. If this type of recombinant is produced, conidiophore formation in this isolate must be induced by near-UV radiation, but conidiophore maturation cannot be suppressed by blue/UV-A radiation, i.e., conidia must develop in continuous irradiation with black light, which contains a near-UV light component effective for conidiation and a blue/UV-A light component effective for suppressing conidiation. However, we were not able to find such isolates either among progenies from crosses examined or in isolates collected in nature. We therefore speculate that this type of recombinant must be easily aborted because of unknown causality.

It was thus suggested that light dependence for conidial development in *B. oryzae* is genetically controlled, and that genes for photo-control of conidiophore induction and genes for photo-control of conidiophore maturation are located on the same chromosome, i.e., they are linked.

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