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# Distribution of photo-induced and non-photo-induced sporulator physiotypes of *Bipolaris oryzae* in Japan

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**The distribution of three sporulator physiotypes of *Bipolaris oryzae*, namely, photo-induced, and non-photo-induced (I) and (II), was investigated. Of 407 isolates, 99% belonged to the photo-induced type, in which conidial development was under photo-control of the antagonistic action of blue/UV-A and near-UV radiation mediated through the 'mycochrome' system at conidiophore induction and conidiophore maturation stages. Of the remainder, 1 isolate belonged to the non-photo-induced (I) type, and 4 isolates belonged to the non-photo-induced (II) type. Conidial development in the former of these was photo-controlled by the 'mycochrome' system at conidiophore maturation stage alone, while in the latter it was not affected by light conditions. No difference was found between the three physiotypes in restriction fragment length polymorphisms (RFLPs) of rDNA. However, random amplified polymorphic DNA (RAPD) revealed polymorphisms between photo-induced and non-photo-induced isolates and showed that non-photo-induced (I) and (II) strains were clustered in the same group, suggesting that they are genetically close. Photo-induced sporulators of *B. oryzae* were confirmed to be widely distributed in paddy fields in Japan.**

Key Words——*Bipolaris oryzae*; mycochrome; photo-control of conidiation; RAPD; RFLP.

It has been reported that there are two sporulator physiotypes of fungi, photo-induced and non-photo-induced, based on the light-dark cycle necessary for induction of conidiophore formation (Leach, 1967; Kumagai, 1978; Tan, 1978). Light is required for the induction of conidiophore formation in the photo-induced sporulators, but not in the non-photo-induced sporulators. It has also been found that conidial development in certain fungi imperfecti such as *Bipolaris oryzae* (Breda de Haan) Shoem., *Alternaria tomato* (Cooke) Weber, *A. cichorii* Nattras, and *Botrytis cinerea* Pers.: Fr. is controlled by an antagonistic action of blue/UV-A and near-UV radiation mediated through the 'mycochrome' system at two developmental stages: induction of conidiophore formation and conidiophore maturation (see Kumagai, 1978; 1988). Conidiophore formation in photo-induced sporulators 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 of photo-induced sporulators 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 applied immediately after blue radiation. The effects of blue and near-UV radiation can be repeatedly reversed, the final response depending on the last type of radiation administered. On the other hand, in a non-photo-induced strain of *B. oryzae*, conidiophore formation can be induced irrespective of light or dark conditions (Yamamura et al., 1978). However, conidial development following conidiophore formation is photo-controlled by the antagonistic action of blue/UV-A and near-UV radiation mediated through the 'mycochrome' system. Recently, we found a new type of non-photo-induced sporulator that differed from the known non-photo-induced (I) sporulators (Kihara and Kumagai, 1994). In this newly identified non-photo-induced (II) sporulator, neither induction of conidiophore formation nor conidial development following conidiophore formation was affected by light conditions. Furthermore, we found that over 95% of 153 isolates collected from rice plants cultivated in paddy fields in Matsue, Shimane Prefecture and in Sendai, Miyagi Prefecture, Japan belonged to the photo-induced physiotype.

The present study aimed to examine the frequency of occurrence of non-photo-induced sporulators of *B. oryzae* in paddy fields in several regions of Japan and to investigate intraspecific polymorphism among the sporulator physiotypes based on RFLP and RAPD analyses using 12 isolates of *B. oryzae* and 1 strain of *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex Dastur, which is morphologically and taxonomically close to *B. oryzae*.

## Materials and Methods

**Experimental strains of *B. oryzae*** Experimental strains of *B. oryzae* (formerly *Helminthosporium oryzae*) were isolated from rice plants cultivated in paddy fields in Miyagi, Akita, Yamagata, and Fukushima Prefectures in

northeastern Japan, Shimane Prefecture in western Japan, and Miyazaki Prefecture in southern Japan in 1992–1996. Several rice leaves with brown lesion spots were detached from rice plants growing in different paddy fields (about $30 \times 30$ m$^2$) located at distances of 1–5 km. Pieces of leaves (each about 25 mm$^2$) with lesion spots were immersed first in 70% (v/v) Et-OH for a few seconds, then in 5% (v/v) sodium hypochloride solution for a few seconds. They were then washed twice with distilled water, put on potato-dextrose agar (PDA) containing chloramphenicol (25 mg/L) with an initial pH of 5.8, and incubated at $25 \pm 1$°C in total darkness for several days, or under darkness for 4 d followed by exposure to black light for 24 h and then darkness for 24 h. A single conidium was microscopically separated from mycelia grown under both light regimes, and these procedures were repeated twice.

*Cochliobolus sativus* (IFO 7502), used as an outgroup of *B. oryzae* for RFLP and RAPD analyses, was purchased from the Institute for Fermentation (Osaka, Japan).

**Light regimes** Experimental strains were grown on PDA at $25 \pm 1$°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. The colonies were flooded with Et-OH, covered with glass covers, and conidia in several areas (each 1 mm$^2$) within the same area of each colony were microscopically ($\times 100$) observed. Black light lamps (FL-20 BLB, Toshiba, Tokyo, Japan), which emitted wavelengths between 320 and 420 nm (mainly 360 nm), were usually used as the source of near-UV radiation for inducing conidiation, the irradiance being about 545 mW m$^{-2}$. Blue light for reversing the inductive effect of near-UV radiation was obtained from colored fluorescent lamps (20 B-F, Toshiba) with emittance of 350 and 550 nm (mainly 460 nm), the irradiance being 368 mW m$^{-2}$.

**DNA extraction** Genomic DNA was extracted from ground lyophilized mycelium grown in potato dextrose broth with shaking for 3–5 d at $25 \pm 1$°C by the protocol of Bruns et al. (1990) with slight modification.

**RFLP analysis of PCR amplified rDNA** Two sets of oligonucleotide primers, NS1 and NS8, and ITS5 and ITS4 (White et al., 1990), were used for polymerase chain reaction (PCR) amplification. These primers amplify specific fragments of the nuclear 18S rDNA, 5.8S rDNA and the internal transcribed spacer (ITS) region between 18S and 28S rDNA (ITS-5.8S-ITS rDNA). Approximately 500 ng of genomic DNA was used in 50 $\mu$l of reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 $\mu$M each of dATP, dCTP, dGTP and dTTP, 1.2 $\mu$M of each primer, and 1.2 U of Taq DNA polymerase (Takara, Otsu, Japan). PCR amplification was performed in a Gene Amp PCR System 9600 (Perkin-Elmer, CT, USA) for 30 cycles of 30 s at 95°C, 30 s at 50°C, and 2 min at 72°C. Amplified DNA fragments were separated by electrophoresis in 1% agarose gel (Takara) with $1 \times$TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The separated fragments were excised from the gel, purified by use of Gene Clean II (BIO 101, La Jolla, CA, USA), and digested with restriction enzymes, *Alu* I, *Rsa* I, *Sau* 3A and *Taq* I (Boeringer Mannheim), *Hae* III, *Hha* I and *Hpa* II (Toyobo, Osaka, Japan), according to the manufacturer's instructions. The digested DNA fragments were separated by electrophoresis in 3% NuSive 3 : 1 GTG agarose (FMC BioProducts, Rockland, ME, USA) with $1 \times$TAE. Gels were stained with ethidium bromide, destained in water, and photographed under UV radiation (360 nm).

**Random amplified polymorphic DNA (RAPD) analysis** RAPD-PCR amplification was performed using a single 10-mer oligonucleotide primer (Operon Technologies, Alameda, CA, USA) and a 12-mer oligonucleotide primer (A08; Wako, Osaka, Japan) by a slight modification of the protocol of Williams et al. (1990). Approximately 200 ng of genomic DNA was used in 20 $\mu$l of reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 $\mu$M each of dATP, dCTP, dGTP and dTTP, 1.2 $\mu$M primer, and 1 U of Taq DNA polymerase (Takara). PCR amplification was performed in a GeneAmp PCR System 9600 (Perkin-Elmer) for 40 cycles of 1 min at 94°C, 1 min at 37°C, and 2 min at 72°C. Amplified DNA fragments were separated by electrophoresis in 1.7% agarose gel (Takara) with $1 \times$TAE. Gels were stained with ethidium bromide, destained in water, and photographed under UV radiation (360 nm).

**Analysis of RAPD data** RAPD data were analyzed to reveal phylogenetic relationships. Genetic similarity was calculated using the Nei and Li (1979) index $S = 2n_{xy}/(n_x + n_y)$, where $n_{xy}$ is the number of shared fragments between two isolates, and $n_x$ and $n_y$ are the numbers of fragments displayed by each isolate. A dissimilarity matrix was computed using the genetic difference index expressed as $M = 1 - S$ (Puterka et al., 1993; van de Zande and Bijlsma, 1995). A dendrogram was constructed from this data matrix by using the unweighted pair group method arithmetic average clustering (UPGMA) analysis with the Neighbor program of PHYLIP 3.53c version (Phylogeny Inference Package, developed by J. Felsenstein, 1991).

## Results

***Grouping of isolates of B. oryzae* into photo-induced and non-photo-induced sporulator physiotypes** The plant pathogenic fungus *B. oryzae* causes brown lesion spots on rice leaves. We collected fungi from typical brown lesion spots caused by *B. oryzae* on rice leaves cultivated in paddy fields in the northeastern, western, and southern regions of Japan (Table 1). Four hundred and seven isolates were identified as *B. oryzae* based on the morphologies of hyphae and conidia grown on PDA and the symptoms of brown lesion spots which developed on detached rice leaves, as compared with those of *B. oryzae* ATCC 38853 (see Kihara and Kumagai, 1994). We first examined the isolates for sporulator physiotype (Table 1). Four hundred and two isolates did not form conidiophores or conidia within 5 d in total darkness or

Table 1. Grouping of isolates of *Bipolaris oryzae* into photo-induced, and non-photo-induced (I) and (II) sporulators.

| Location | Year | No. of strains tested | Type of sporulator | | |
|---|---|---|---|---|---|
| | | | Photo-induced | Non-photo-induced (I) | Non-photo-induced (II) |
| Shimane | 1992[a)] | 142 | 137 | 1 | 4 |
| | 1993 | 132 | 132 | 0 | 0 |
| Miyagi | 1992[a)] | 11 | 11 | 0 | 0 |
| | 1994 | 76 | 76 | 0 | 0 |
| | 1996 | 8 | 8 | 0 | 0 |
| Akita | 1994 | 4 | 4 | 0 | 0 |
| Yamagata | 1994 | 21 | 21 | 0 | 0 |
| Fukushima | 1994 | 7 | 7 | 0 | 0 |
| Miyazaki | 1994 | 6 | 6 | 0 | 0 |
| Total | | 407 | 402 | 1 | 4 |

a) Reported by Kihara and Kumagai (1994).

under continuous exposure to blue light. When the isolates were grown under black light, conidiophores were produced in profusion at all loci on the colony, although no conidia were formed. When they were transferred into darkness for 1 d following the exposure to black light for 4 d, all conidiophores produced conidia at all loci on the colony. However, when blue radiation for 1 d was applied after the exposure to black light, conidiophores differentiated into 'sterile' ones, and no conidia were formed. It was thus confirmed that these 402 isolates

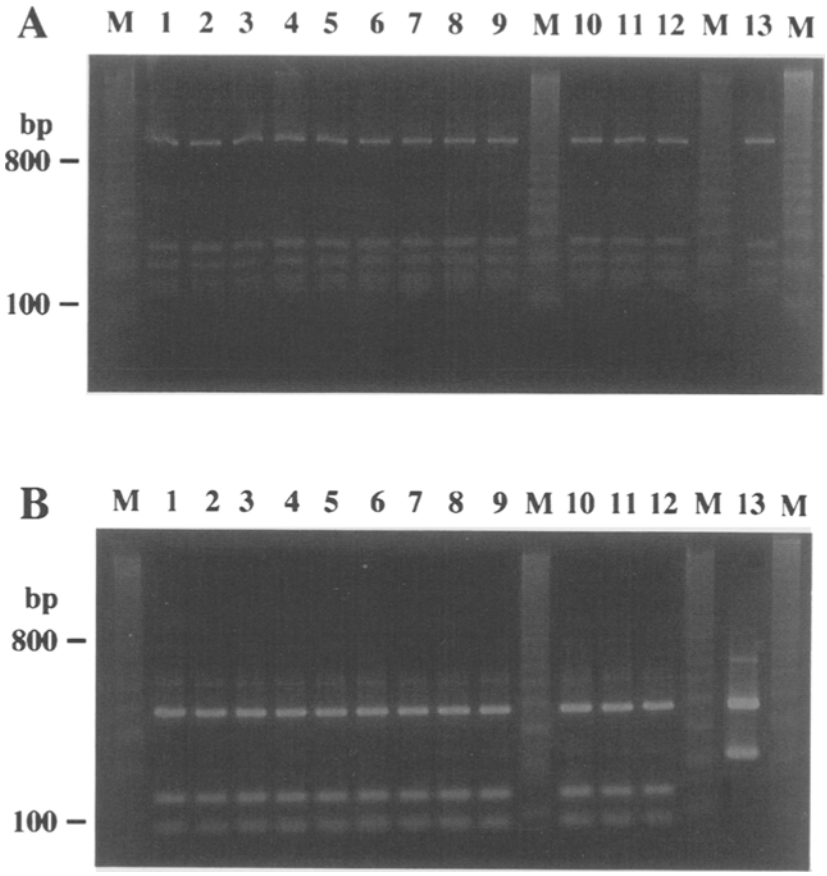


Fig. 1. Representative RFLP patterns of PCR-amplified 18S rDNA digested with *Hha* I (A) and ITS-5.8S-ITS rDNA digested with *Rsa* I (B).
Lane M, 100 bp ladder marker; lanes 1–9, ATCC38853, isolates B4, B23, B38, S17-2, T2-90, M1, F6, K6, respectively, photo-induced sporulator of *B. oryzae*; lane 10, isolate D6, non-photo-induced (I) sporulator of *B. oryzae*; 11–12, isolates D3 and D9, respectively, non-photo-induced (II) sporulator of *B. oryzae*; lane 13, *C. sativus*.

could be classified as photo-induced sporulators.

The remaining five isolates, D1, D3, D5, D6 and D9, formed conidiophores with conidia in total darkness; these isolates belonged to the non-photo-induced sporulator physiotype. When one of these isolates, D6, was grown under continuous exposure to black light, only conidiophores were formed, as in the case of photo-induced sporulators. However, when cultures were transferred into darkness for 1 d following exposure to black light for 4 d, conidia were produced in profusion at all loci on the colony. When cultures were grown under blue radiation following the exposure to black light, conidiophores dedifferentiated into 'sterile' ones, and conidia did not form. That is, conidial development in this fungus was under the photo-control of the antagonistic action of blue and near-ultraviolet radiation at conidiophore maturation stage, but not at the conidiophore induction stage; this isolate was classified as a non-photo-induced (I) sporulator. The other four isolates, D1, D3, D5 and D9, formed conidiophores with conidia irrespective of light conditions. They appeared to have lost the photo-regulation of conidial development by the 'mycochrome' system at both developmental stages of conidiophore induction and conidiophore maturation; these isolates were classified as non-photo-induced (II) sporulators.

It is thus evident that photo-induced strains of *B. oryzae*, which are under the photo-control of antagonistic action of blue/UV-A and near-UV radiation mediated through the 'mycochrome' system, are widely distributed in paddy fields in Japan. Two different types of non-photo-induced sporulators of the fungus were found, although they were few.

**Analyses of rDNA-RFLP and RAPD-PCR** We examined the extent to which the photo-induced and non-photo-induced (I) and (II) strains differed from each other at the molecular level in two respects: restriction fragment length polymorphisms (RFLPs) in the PCR amplified fragments of 18S rDNA and ITS-5.8S-ITS rDNA, and random amplification of polymorphic DNA (RAPD). The twelve isolates shown in Fig.1 were selected for the experiments. *Cochliobolus sativus* was also examined. The PCR amplified fragments were separately digested with seven restriction enzymes, *Alu* I, *Rsa* I, *Sau* 3A, *Taq* I, *Hae* III, *Hha* I, and *Hpa* II, and electrophoresed. All RFLP banding patterns of 18S rDNA and ITS-5.8S-ITS rDNA digested with the seven restriction enzymes were similar for the tested strains of *B. oryzae*, while polymorphism appeared in *C. sativus* (data not shown). The RFLP banding patterns of 18S rDNA digested with *Hha* I (Fig .1 A) and ITS-5.8S-ITS rDNA digested with *Rsa* I (Fig. 1 B) of the tested strains are shown by way of example.

Next, RAPD analysis was performed. Seventy-two unique primers were employed in an initial screening of *B. oryzae*, and the following 5 primers were selected for detailed analysis: A08, GCCCCGTTAGCA (Wako); OPB04, GGACTGGAGT; OPB06, TGCTCTGCCC; OPJ-01, CCCGGCATAA; and OPJ06, TCGTTCCGCA (Operon Technologies). The RAPD banding patterns of tested strains of *B. oryzae* and *C. sativus* generated by primers OPJ01 and OPB06 are shown in Fig. 2; the other 3

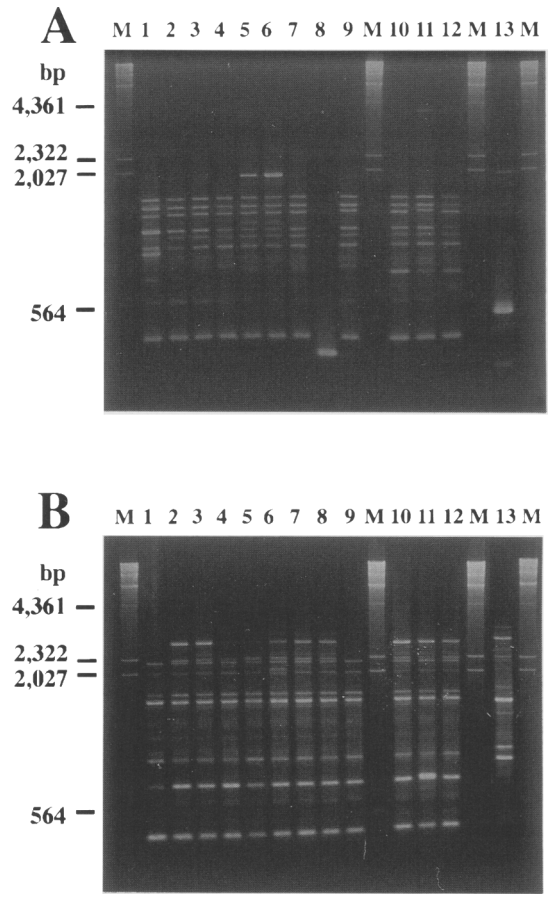


Fig. 2. Random amplified polymorphic DNA profile of 12 isolates of *B. oryzae* and *C. sativus* produced by two primers, OPJ 01 (A) and OPB06 (B).
Lane M, *Hin*dIII cut lamda DNA; lanes 1–9, ATCC38853, isolates B4, B23, B38, S17-2, T2-90, M1, F6, K6, respectively, photo-induced sporulators of *B. oryzae*; lane 10, isolate D6, non-photo-induced (I) sporulator of *B. oryzae*; 11–12, isolates D3 and D9, respectively, non-photo-induced (II) sporulators of *B. oryzae*; lane 13, *C. sativus*.

primers also amplified reproducible patterns, as did OPJ01 and OPB06.

It was thus confirmed that all tested isolates could be classified as *B. oryzae* on the basis of rDNA-RFLP, although there were intraspecific polymorphisms within isolates of *B. oryzae* on the basis of RAPD.

**Phylogenetic relationships of isolates of *B. oryzae*** Phylogenetic relationships among photo-induced and non-photo-induced isolates of *B. oryzae* together with *C. sativus* were investigated based on the data of RAPD experiments. Genetic difference based on the 40 polymorphic DNA bands scored were calculated for each pair of the strains of *B. oryzae* and *C. sativus* (Table 2). The relationships between the isolates of *B. oryzae* were represented by construction of a dendrogram from the genetic dissimilarity matrix data (Table 2) using the unweighted pair group method with arithmetic average clustering (UPGMA) analysis. The dendrogram revealed many variations at the DNA level between isolates of *B.*

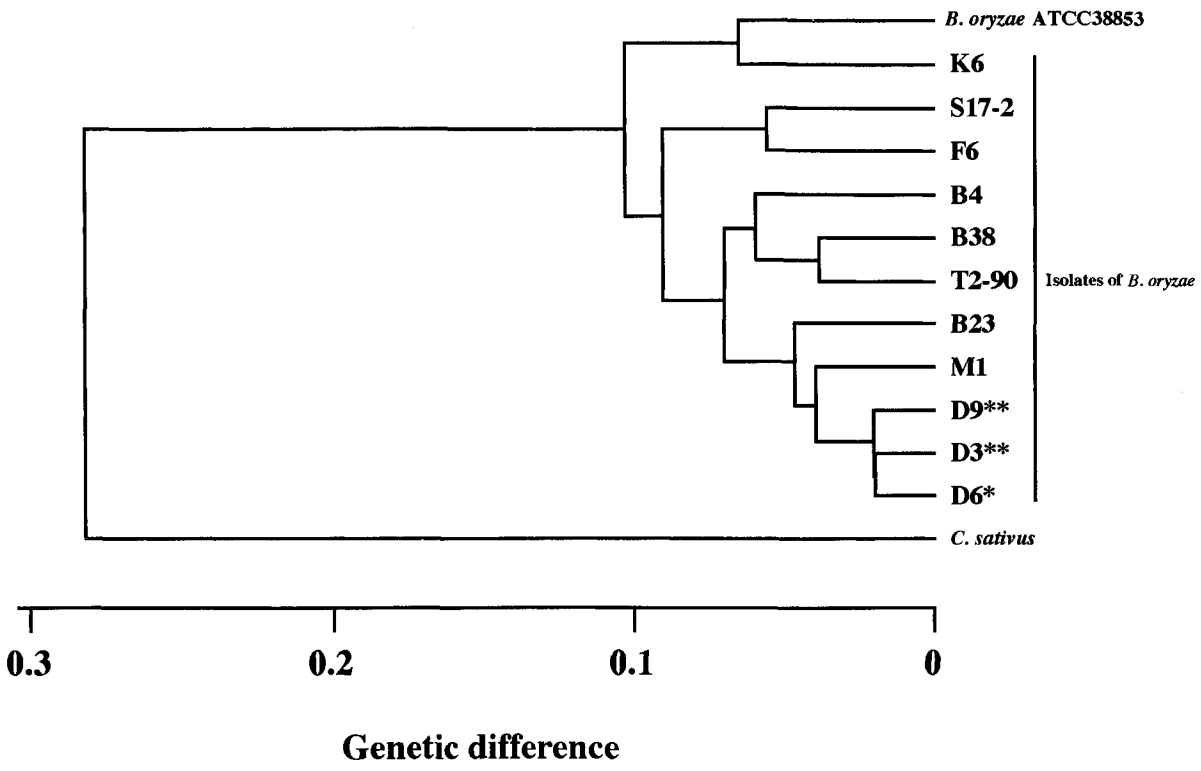


Fig. 3. UPGMA cluster dendrogram of genetic differences calculated from RAPD data using primers OPB 04, OPB 06, OPJ 01, OPJ 06 and A08 for *B. oryzae* isolates and *C. sativus*.
The extent of genetic difference is indicated by the scale at the bottom of the diagram, which ranges from 0, when all individuals exhibit the same divergence, to 1, when each individual is unique. * and ** show non-photo-induced (I) and (II) isolates of *B. oryzae*, respectively.

Table 2. *Dissimilarity matrix based on genetic differences for each pair of strains of B. oryzae and C. sativus by RAPD analysis using five different primers.*

| | ATCC 38853 | B4 | B23 | B38 | S17-2 | T2-90 | M1 | F6 | K6 | D6 | D3 | D9 | *C. sativus* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ATCC38853 | | | | | | | | | | | | | |
| B4 | 0.184 | | | | | | | | | | | | |
| B23 | 0.240 | 0.143 | | | | | | | | | | | |
| B38 | 0.154 | 0.137 | 0.154 | | | | | | | | | | |
| S17-2 | 0.265 | 0.208 | 0.143 | 0.176 | | | | | | | | | |
| T2-90 | 0.160 | 0.102 | 0.120 | 0.077 | 0.143 | | | | | | | | |
| M1 | 0.236 | 0.148 | 0.091 | 0.158 | 0.111 | 0.127 | | | | | | | |
| F6 | 0.304 | 0.200 | 0.174 | 0.250 | 0.111 | 0.217 | 0.216 | | | | | | |
| K6 | 0.130 | 0.156 | 0.174 | 0.167 | 0.244 | 0.130 | 0.216 | 0.286 | | | | | |
| D6 | 0.231 | 0.176 | 0.077 | 0.148 | 0.137 | 0.115 | 0.053 | 0.250 | 0.167 | | | | |
| D3 | 0.200 | 0.143 | 0.080 | 0.115 | 0.102 | 0.080 | 0.091 | 0.217 | 0.130 | 0.038 | | | |
| D9 | 0.240 | 0.184 | 0.120 | 0.154 | 0.102 | 0.120 | 0.091 | 0.217 | 0.174 | 0.038 | 0.040 | | |
| *C. sativus* | 0.676 | 0.500 | 0.568 | 0.590 | 0.556 | 0.514 | 0.524 | 0.515 | 0.697 | 0.538 | 0.568 | 0.514 | |

*oryzae* and that the cluster of *B. oryzae* was clearly distinct from that of *C. sativus* (Fig. 3). It is also notable that non-photo-induced isolates (D3, D6 and D9) clustered in the same group, which was separate from the photo-induced isolates.

## Discussion

As stated previously, conidial development in photo-induced sporulators such as *A. tomato*, *A. cichorii*, *B. oryzae*, and *B. cinerea* is photo-controlled by the 'mycochrome' system at the conidiophore induction and

conidiophore maturation stages, while that in a non-photo-induced (I) strain of *B. oryzae* is photo-controlled at the conidiophore maturation stage alone. We recently found a new type of non-photo-induced (II) sporulator, in which conidial development is unaffected by light conditions. In this study, we investigated the geographical distribution of each sporulator physiotype of *B. oryzae* by analyzing the light dependency of conidial development of isolates collected from brown lesion spots on rice leaves cultivated in paddy fields in Japan. We found that the photo-induced sporulator physiotype accounts for about 99% of 407 isolates of *B. oryzae* and was widely distributed in Japan (Table 1). On the other hand, only a few non-photo-induced strains were isolated, all of which were found in Matsue, Shimane Prefecture, in 1992. No such strains were found among these isolated from paddy fields in the locations listed in Table 1 in 1994–1996. These results suggest that the photo-induced strains of *B. oryzae* might be more adaptable to nature than the non-photo-induced strains, and that the latter might be spontaneous mutants of the former.

No differences were found between photo-induced and non-photo-induced isolates in morphologies of hyphae and conidia or in symptoms of brown lesion spots developed on the detached rice leaf (data not shown; see Kihara and Kumagai, 1994). We therefore attempted to investigate whether or not these sporulator physiotypes were distinguishable at the genomic DNA level.

It has been reported that RFLP analysis of rDNA is a useful technique for identification of some fungi (Cubeta et al., 1991; Chen, 1992; Ward and Akrofi, 1994; Erland, 1995), and that RAPD analysis is also useful for identification of fungi (Bentley et al., 1995; Theodore et al., 1995) and for investigation of the correlation of genetic variation with pathotype (Nicholson and Rezanoor, 1994). Nakada et al. (1994) have reported that analysis of RFLPs of total DNA using arbitrarily chosen genomic clones as probe revealed clear differences among the most common plant pathogenic fungi, *Bipolaris* and *Curvularia* species, on gramineous plants. Twelve isolates of *B. oryzae* belonging to photo-induced and non-photo-induced (I) and (II) sporulators were selected for analyses of RFLP and RAPD. These were compared with each other and with *C. sativus*, which is morphologically and taxonomically close to *B. oryzae*. No intraspecific variation was detectable among the isolates of *B. oryzae* in RFLP analysis of rDNA (Fig. 1), indicating that photo-induced and non-photo-induced isolates of *B. oryzae* are very close to each other at the nuclear rDNA level. On the other hand, polymorphisms were found among isolates of *B. oryzae* (Fig. 2) in RAPD analysis, and the cluster of non-photo-induced isolates in the dendrogram of RAPD analysis formed a separate group from the photo-induced isolates (Fig. 3). It was thus suggested that the non-photo-induced (I) and (II) sporulators were genetically close to each other.

Intraspecific polymorphisms also appeared among photo-induced isolates and among non-photo-induced isolates, even though the genetic differences between these isolates were smaller. In our previous work (Kihara and Kumagai, 1994), different photo-sensitivities in conidial development of photo-induced isolates of *B. oryzae* were detected. It appears that the fungus *B. oryzae* might genetically vary in nature, and that light dependency of its conidial development might also vary. Furthermore, it was found that the photo-induced sporulator accounted for the majority of isolates collected from rice plants in various paddy fields. It is unclear whether photo-induced strains are more adaptable to the present environment than non-photo-induced strains, however, and this problem should be studied further.

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## Literature cited

Bentley, S., Pegg, K. G. and Dale, J. L. 1995. Genetic variation among a world-wide collection of isolates of *Fusarium oxysporum* f. sp. *cubense* analyzed by RAPD-PCR fingerprinting. Mycol. Res. **99**: 1378–1384.

Bruns, T. D., Fogel, R. and Taylor, J. W. 1990. Amplification and sequencing of DNA from fungal herbarium specimens. Mycologia **82**: 175–184.

Chen, W. 1992. Restriction fragment length polymorphisms in enzymatically amplified ribosomal DNAs of three heterothallic *Pythium* species. Phytopathology **82**: 1467–1472.

Cubeta, M. A., Echandi, E., Abernethy, T. and Vilgalys, R. 1991. Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. Phytopathology **81**: 1395–1400.

Erland, S. 1995. Abundance of *Tylospora fibrillosa* ectomycorrhizas in a South Swedish spruce forest measured by RFLP analysis of the PCR-amplified rDNA ITS region. Mycol. Res. **99**: 1425–1428.

Kihara, J. and Kumagai, T. 1994. Ecotypes of the fungus *Bipolaris oryzae* with various responses of the mycochrome system. Physiol. Plant. **92**: 689–695.

Kumagai, T. 1978. Mycochrome system and conidial development in certain fungi imperfecti. Photochem. Photobiol. **27**: 371–379.

Kumagai, T. 1988. Photocontrol of fungal development. Photochem. Photobiol. **47**: 889–896.

Leach, C. M. 1967. Interaction of near-ultraviolet light and temperature on sporulation of the fungi, *Alternaria, Cercosporella, Fusarium, Helminthosporium,* and *Stemphylium*. Can. J. Bot. **45**: 1999–2016.

Nakada, M., Tanaka, C., Tsunewaki, K. and Tsuda, M. 1994. RFLP analysis for species separation in the genera *Bipolaris* and *Curvularia*. Mycoscience **35**: 271–278.

Nei, M. and Li, W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. **76**: 5269–5273.

Nicholson, P. and Rezanoor, H. N. 1994. The use of random amplified polymorphic DNA to identify pathotype and detect variation in *Pseudocercosporella herpotrichoides*. Mycol. Res. **98**: 13–21.

Puterka, G. J., Black, W. C., Steiner, W. M. and Burton, R. L. 1993. Genetic variation and phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), inferred from allozyme and RAPD-PCR markers. Heredity **70**: 604–618.

Tan, K. K. 1978. Light-induced fungal development. In: The filamentous fungi, vol. III. Developmental biology, (ed. by Smith , J. E. and Berry, D. R.), pp. 334–357. Edward Arnold Publ., London.

Theodore, M. L., Stevenson, T. W., Johnson, G. C., Thornton, J. D. and Lawrie, A. C. 1995. Comparison of *Serpula lacrymans* isolates using RAPD PCR. Mycol. Res. **99**: 447–450.

Van de Zande, L. and Bijlsma, R. 1995. Limitations of the RAPD technique in phylogeny reconstruction in *Drosophila*. J. Evol. Biol. **8**: 645–656.

Ward, E. and Akrofi, A. Y. 1994. Identification of fungi in the *Gaeumannomyces-Phialophora* complex by RFLPs of PCR-amplified ribosomal DNAs. Mycol. Res. **98**: 219–224.

White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: A guide to methods and amplifications, (ed. by Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J.), pp. 315–322. Academic Press, San Diego.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuclic Acids Res. **18**: 6531–6535.

Yamamura, S., Kumagai, T. and Oda, Y. 1978. Mycochrome system and conidial development in a nonphotoinduced isolate of *Helminthosporium oryzae*. Can. J. Bot. **56**: 206–208.