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Diversity of SCAR Markers of *Pyricularia grisea* Isolated from *Digitaria ciliaris* Following Cross Infection to Rice	Sri Listiyowati, Utut Widyastuti, Gayuh Rahayu, Alex Hartana, and Muhammad Jusuf	1
Glucose Biosensor Using Selected Indonesian Bacteria	Dyah Iswantini, Novik Nurhidayat, and Trivadila	9
Screening of Actinomycetes Producing an ATPase Inhibitor of *Japanese Encephalitis Virus* RNA Helicase from Soil and Leaf Litter Samples	Shanti Ratnakomala, Roni Ridwan, Puspita Lisdiyanti, Abinawanto, and Andi Utama	15
Genome-Shuffling-Improved Acid Tolerance and Lactic Acid Production in *Lactobacillus plantarum* for Commercialization	Lita Triratna, Budi Saksono, Linda Sukmarini, and Asep Suparman	21
Optimization of Human Interferon α2b Soluble Protein Overproduction and Primary Recovery of Its Inclusion Bodies	Ratih Asmana Ningrum, Debbie Sofie Retnoningrum, Yeyet Cahyati, and Heni Rachmawati	27
Ultrastucture of *Wolbachia* are Found in Somatic and Reproductive Tissue of *Drosophila simulans* and *D. melanogaster*	Endang Srimurni Kusmintarsih	33
Process Design of Microbiological Chitin Extraction	Budiasih Wahyuntari, Junianto, and Siswa Setyahadi	39
Screening of Quorum Quenching Activity of Bacteria Isolated from Ant Lion	Billy Christianto and Yogiara	46

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Diversity of SCAR Markers of *Pyricularia grisea* Isolated from *Digitaria ciliaris* Following Cross Infection to Rice

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Cross infection of *Pyricularia grisea* from grass to rice and *vice versa* has been reported, but genetic changes are not known yet. This research aimed at estimating the possibility of the genotype alteration in *P. grisea* dc4 isolated from *Digitaria ciliaris*, following cross infection to either rice cv. Kencana bali, Cisokan, and IR64 or *Panicum repens, Cynodon dactylon, Digitaria* sp., and *Ottochloa nodosa*. The genotypes were analyzed by employing three SCAR markers, Cut1; PWL2; and Erg2. The results indicated that the dc4 was only able to infect Kencana bali, Cisokan, and *P. repens*. The dc4 had only two out of three SCAR markers, Cut1 and Erg2. Host shift was followed by genotype alteration in two loci of SCAR. Isolates derived from lesions on Kencana bali (dc4-kb) and Cisokan (dc4-c) of the dc4 infection, both lost their Cut1 and gained PWL2. On the contrary, there was no genotype alteration from dc4 to isolate derived from *P. repens* of dc4 infection (dc4-pr). Neither the isolate dc4-kb that was cross-inoculated to Cisokan nor the dc4-c that was cross-inoculated to Kencana bali showed SCAR marker change. In comparison, race 173 isolate and those derived from Kencana bali and Cisokan did not show genotype alteration. All had two out of three SCAR markers, PWL2 and Erg2. The isolate 173 was adapted to rice. This indicated that genotype diversity of the dc4 might arise following host shift from grass to rice.

Key words: *Pyricularia grisea, Digitaria ciliaris*, rice, *Panicum repens*, SCAR markers

Pyricularia grisea merupakan cendawan blas yang telah diketahui memiliki kisaran inang luas selain pada padi. Infeksi silang cendawan blas pada rumput ke padi dan sebaliknya telah dilaporkan, tetapi perubahan genetiknya belum dilaporkan. Tujuan penelitian ini menganalisis kemampuan infeksi silang dan perubahan genotipe *P. grisea* dc4 asal *Digitaria ciliaris* dalam perpindahannya ke padi cv. Kencana bali, Cisokan, dan IR64 atau rumput *Panicum repens, Cynodon dactylon, Digitaria* sp. dan *Ottochloa nodosa*. Genotipe *P. grisea* dianalisis melalui tiga marka SCAR, yaitu Cut1; PWL2; dan Erg2. Isolat dc4 memiliki 2 marka SCAR, yaitu Cut1 dan Erg2; tidak memiliki PWL2. Isolat dc4 hanya mampu menginfeksi silang Kencana bali, Cisokan, dan *P. repens*. Turunan isolat dc4 sebagai hasil infeksi silang ke Kencana bali (dc4-kb) dan Cisokan (dc4-c) menunjukkan perubahan genotipenya, yaitu Cut1 tidak teramplifikasi pada keduanya; PWL2 teramplifikasi;, serta Erg2 tetap teramplifikasi. Sebaliknya, turunan isolat dc4 sebagai hasil infeksi silang ke *P. repens* (dc4-pr) tidak mengalami perubahan genotipe. Turunan isolat dc4-kb sebagai hasil infeksi silang ke Cisokan, maupun turunan isolat dc4-c dari Kencana bali, juga tidak menunjukkan perubahan genotipe, yaitu tetap menunjukkan keberadaan PWL2 dan Erg2. Sebagai pembanding digunakan isolat ras 173 yang diisolasi dari padi. Genotipe isolat tersebut maupun turunannya, sebagai hasil infeksi silang ke Kencana bali dan Cisokan, tidak menunjukkan perubahan. Perubahan genotipe dc4 terjadi mengikuti pergantian inang dari rumput ke padi.

Kata kunci: *Pyricularia grisea, Digitaria ciliaris*, padi, *Panicum repens*, marka SCAR

Pyricularia grisea (teleomorph *Magnaporthe grisea* is known as a rice blast causal agent (Rossman *et al.* 1990). Grasses and cereals can serve as hosts to *P. grisea*, even though not all isolates of *P. oryzae* are pathogenic to rice cultivars (Mackill and Bonman 1986; Singh and Singh 1988; DBPT 1992) or other *Graminae* (Tredway *et al.* 2005). Transmission to different hosts has been reported to occur in many fungal pathogens. This needs the capability to adapt to new environment. Therefore, host shift might cause diversity in *P. grisea* and determine the genetic structure of that population.

Various weed grasses, such as *Echinochloa crusgalli, Leersia hexandra, Panicum repens*, and *P. maximum,* which grew surrounding the rice field, have been reported to host *P. grisea* in Indonesia (DBPT 1992). In 2005, the blast lesions were found on *Digitaria ciliaris*, *Digitaria* sp., and *Ottochloa nodosa* in experimental rice fields in Jasinga, Bogor, as well as on *Cynodon dactylon* and *P. repens* in Sukabumi. The isolates from grasses of *Echinochloa colona, L. hexandra,* and *Rottboellia exaltata* potentially produced blast symptoms on blast-susceptible rice cultivars and *vice versa* (Mackill and Bonman 1986).

Various fingerprinting molecular markers have been developed to analyze the genetic structure and population dynamics of *P. grisea*. New developed markers that are commonly used to analyze the diversity of *P. grisea* are the sequenced characterized amplified region (SCAR) markers. The SCAR technique is a simple PCR-based method. Thus, SCAR

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markers are more reproducible than fingerprinting molecular markers. Sixteen SCAR markers have been used to survey the genetic diversity within *P. grisea* population (Soubabere *et al.* 2001). Three SCAR markers, i.e. Cut1; PWL2; and Erg2; were able to determine the haplotype variability of rice blast pathogen originated from endemic areas of blast disease (Reflinur *et al.* 2005).

The objective of this research was to study the effect of host shift on the genotypic diversity of *P. grisea* originated from *D. ciliaris,* and to obtain information on the relationship between *P. grisea* from *D. ciliaris* and rice. Thus, this information will broaden the understanding on factors affecting genetic structure changes in *P. grisea*, and will be used as the basis to set up recommendation on blast disease management.

MATERIALS AND METHODS

Fungal Strains. Mono-conidial isolate dc4 was obtained from the blast symptom on *D. ciliaris* that grew in experimental rice fields in Jasinga, Bogor in 2005. *P. grisea* race 173 was isolated from an unknown rice cultivar by Anggiani Nasution, Balitpa Bogor. This strain was used as a comparative isolate.

Plants for Cross-Infection Test. Three differential rice cultivars of *P. grisea* race 173 (cv. Kencana bali, Cisokan, and IR64), healthy grasses *Panicum repens* and *Cynodon dactylon* (collected from blast endemic area in Sukabumi) and *Digitaria* sp., and *Ottochloa nodosa* (obtained from Jasinga, Bogor, where *Digitaria ciliaris* showed blast symptoms) were used as plant materials.

Isolation and Culture Maintenance. To obtain mono-conidial culture of *P. grisea* dc4 and from each step of the cross-infection test, single lesions on a leaf were first cut off, washed under running water, and then put in moistened sterilized Petri dish overnight at room temperature. The lesion was observed under a microscope, and the conidia on the lesion were streaked onto a thin layer of water agar 4% (w/v). Individual conidia were isolated using method of Bonman *et al.* (1986) and aseptically transferred and cultured. All cultures were maintained in Potato Dextrose Agar slants (Difco).

Inoculum Preparation. Conidia were used as a source of inoculum. The isolates were grown on oatmeal agar (oat 30 g L^{-1}, agar 20 g L^{-1}, sucrose 5 g L^{-1}) in Petri dishes and incubated at room temperature for 7 d until the entire agar surface was covered with mycelial growth. The culture was then aseptically washed to remove the aerial hyphae. The culture was continually exposed under n-UV rays for 4-5 d. About 3 mL of sterilized water containing 0.025% (v/v) Tween 20 was poured onto the fungal colony. The colony surface was rubbed with a sterile object glass to collect the conidia. The suspension was filtered through a cheesecloth, and the concentration of conidia was estimated with a haemocytometer, then adjusted to 10^4-10^5 conidia mL^{-1} (Luo *et al.* 2004) with some modifications, i.e. medium composition, Tween concentration, and conidial suspension filter.

Cross-Infection Test with Artificial Inoculation. In order to obtain *P. grisea* isolate that had gone through host shift, two steps of artificial infection were conducted. The method for leaf blade infection refers to Tanabe *et al.* (2006). In the first step, 1 mL suspension of *P. grisea* dc4 conidia (10^4-10^5 conidia mL^{-1}) was inoculated by syringe on the 4th or 5th sheath of the 3 cultivars of rice (16 d old seedling) and 4 grasses (16 d old after replanting). They were placed in plastic pots containing 1 kg of soil fertilized with compost (1:1). All of the treated plants were incubated in a humid and dark condition for 2 x 24 h, and then transferred out into a light room for 6 d at 25-30 °C and 70-80% humidity (Silue *et al.* 1992). Observation on blast lesions was made at 4 to 9 d after inoculation. Cross infection was determined qualitatively (Tanabe *et al.* 2006) with modification of infected criteria. The infection was positive when the infection produced blast lesion on the leaf blade and the sporulation had to be detected on that lesion after keeping it in moistened sterilized Petri dish overnight at room temperature. On the contrary, it was negative when artificial infection did not produce blast lesion on the leaf blade.

A single conidium from the moistened lesions was re-isolated. The culture was designated as dc4-host1, and the code of host varies depending on the host used. The second step, conidia of dc4-host1 culture was used as a source inoculum for further artificial inoculation to a range of host. Re-isolation was done with a similar method and the culture was designated as dc4-host1-host2.

The artificial inoculation was also done for *P. grisea* race 173 to a series of rice cultivars and grasses, but no further artificial inoculation to rice cultivars. As negative control, rice plants and 4 grasses were inoculated with aquadest. Three repetitions were prepared for each isolate. All cultures were obtained from the first and second steps of artificial infection and the original culture was used as a source of DNA.

SCAR Analysis. Molecular analysis was performed on one of three mono-conidial cultures obtained from a single lesion of *P. grisea*. Genomic DNA was extracted from mycelia grown in 25 mL

liquid medium (sucrose 5 g L^{-1}, yeast extract 2 g L^{-1}, and peptone 2 g L^{-1}) for 6 d on a shaker. The mycelia were harvested by filtering using filter paper. DNA isolation was conducted using the method described by Raeder and Broda (1985) with modifications, i.e. cultural liquid-medium type and centrifugation speed. Mycelia were ground in a sterile mortar, and then suspended in 4 mL of extraction buffer solution (200 mM Tris HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS). A solution of 2.8 mL phenol and 1.2 mL CIA (chloroform:isoamil alcohol = 24:1) was added to the suspension and then mixed by gently shaking it back and forth. The suspension was centrifuged at 4000 rpm and 6 °C for 30 min. The upper aqueous phase was transferred right away into a new tube and precipitated by adding 1 vol cold isopropanol. Next it was centrifuged at 4000 rpm and 6 °C for 20 min. The precipitation was rinsed with cold ethanol 70% and then re-centrifuged for 10 min. After that, the pellet was vacuum-dried for 15 min, and dissolved in 100 µL TE 1x (10 mm Tris HCl pH 8, 1 mmol EDTA), and then 0.2 vol RNAse 20 mg mL^{-1} was added. The solution was incubated overnight at 37 °C, then 900 µL TE 1x was added, and the solution was again extracted by adding 1 vol CIA, and centrifuged at 4000 rpm and 6 °C for 10 min. The upper liquid was precipitated again by adding cold isopropanol as in previous steps.

The isolated DNA, after quantified and diluted, would then be amplified through PCR using three primers developed by Soubabere *et al.* (2001), i.e. Cut1; PWL2; and; Erg2. The nucleotide sequences of the three primers are as follows:

Cut1(F:5'-TATAGCGTTGACCTTGTGGA-3')
Cut1(R:5'-TAAGCATCTCAGACCGAACC-3')
PWL2(F:5'-TCCGCCACTTTTCTCATTCC-3')
PWL2(R: 5'-GCCCTCTTCTCGCTGTTCAC-3')
Erg2 (F:5'-GCAGGGCTCATTCTTTTCTA-3')
Erg2 (R:5'-CCGACTGGAAGGTTTCTTTA-3')

The total PCR reaction of 20 µL contained around 100 ηg genomic DNA template, 10 µL of 2xPCR master mix (0.05 unit µL^{-1} Taq DNA polymerase, 4 mM MgCl, 0.4 mM from each dNTP), and 0.6 ρmol from each primer. The PCR program consisted of pre-denaturation at 95 °C for 15 min, followed by 35 cycles at 94 °C for 15 sec, at 60 °C for 30 sec, and at 72 °C for 1 min. The last step was at 72 °C for 7 min. PCR results were visualized through electrophoresis on agarose gel 1% (w/v) in TAE 1x, and continued with gel immersion in ethidium bromide (0.5 µg mL^{-1}). Evaluation was conducted based on the presence of DNA band on the agarose gel.

RESULTS

Cross Infection Ability. *Pyricularia grisea* dc4 was able to infect Kencana bali (Fig 1a), Cisokan (Fig 1b), and *P. repens* (Fig 1c), but was unable to infect IR64, *C. dactylon*, *Digitaria* sp., and *O. nodosa* (Table 1). Strain dc4-kb of Kencana bali might infect Cisokan, and dc4-c of Cisokan was able to infect Kencana bali as well. Strain dc4-kb might also infect

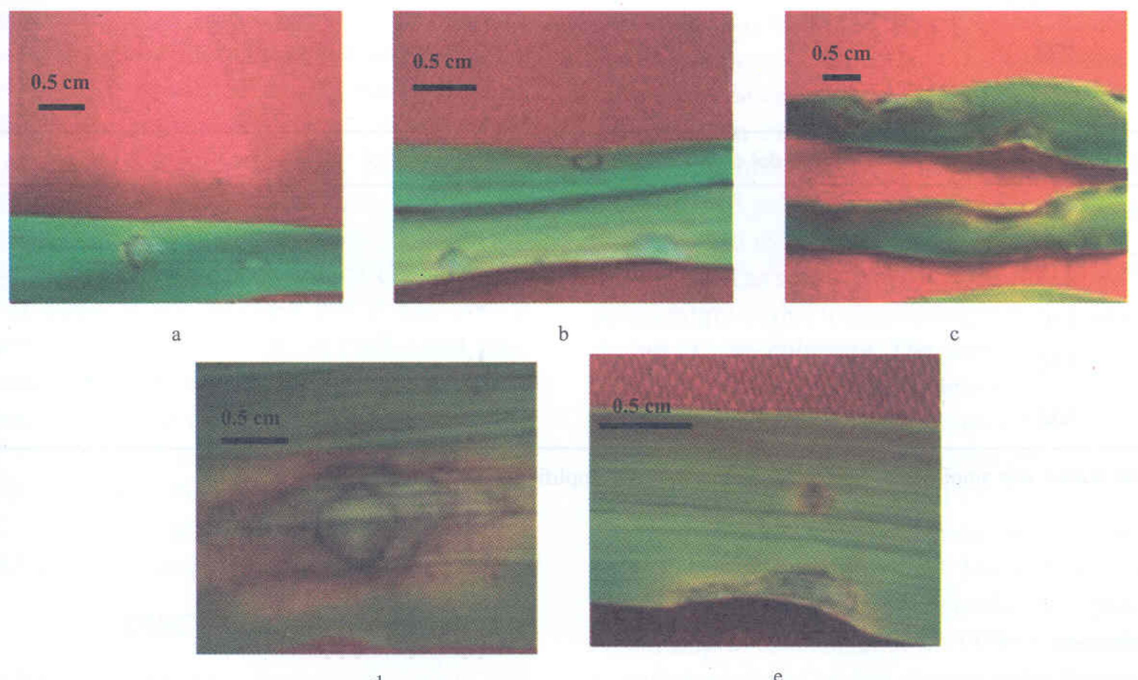

Fig 1 Blast lesions on leaves of rice cultivars. a, Kencana bali; b, Cisokan; c, *Panicum repens* on day 9 after was inoculated isolate *Pyricularia grisea* dc4 from *Digitaria ciliaris*; d, blast lesions on rice leaves of Kencana bali; and e, Cisokan on day 7 after was inoculated *P. grisea* race 173 from rice.

Table 1 Inoculation results of isolate *Pyricularia grisea* dc4 from *Digitaria ciliaris* and race 173 from rice on Kencana bali, Cisokan, IR64, *Panicum repens*, *Cynodon dactylon*, *Digitaria* sp., and *Ottochloa nodosa*; as well as SCAR markers of isolates *P. grisea* dc4 and the first cross infection

	Strain isolates of inoculum	Plants of first cross infection test						
	P. grisea dc4 from *D. ciliaris*	Kencana bali	Cisokan	IR64	*P. repens*	*C. dactylon*	*Digitaria* sp.	*O. nodosa*
Inoculation results		lesion	lesion	no lesion	lesion	no lesion	no lesion	no lesion
Re-isolates		dc4-kb	dc4-c		dc4-pr			
SCAR Markers: Cut1	+	-	-		+			
PWL2	-	+	+		-			
Erg2	+	+	+		+			
	P. grisea race 173	Kencana bali	Cisokan	IR64	*P. repens*	*C. dactylon*	*Digitaria* sp.	*O. nodosa*
Inoculation results		lesion	lesion	no lesion	no lesion	no lesion	no lesion	no lesion
Re-isolates		173-kb	173-c					
SCAR Markers: Cut1	-	-	-					
PWL2	+	+	+					
Erg2	+	+	+					

+, molecular marker was amplified; -, molecular marker was not amplified.

Table 2 Inoculation results of *P. grisea* dc4-kb and dc4-c on Kencana bali, Cisokan, and *P. repens*; as well as SCAR markers of the second infection

	Strain isolates of inoculum	Plants of cross infection test		
	P. grisea dc4-kb	Kencana bali	Cisokan	*P. repens*
Inoculation results		lesion	lesion	lesion
Re-isolates		dc4-kb-kb	dc4 -kb-c	NI
SCAR markers: Cut1	-	-	-	
PWL 2	+	+	+	
Erg2	+	+	+	
	P. grisea dc4-c	Kencana bali	Cisokan	*P. repens*
Inoculation results		lesion	lesion	no lesion
Re-isolates		dc4-c-kb	dc4-c-c	
SCAR markers: Cut1	-	-	-	
PWL 2	+	+	+	
Erg2	+	+	+	

+, molecular marker was amplified; -, molecular marker was not amplified; NI, no identified.

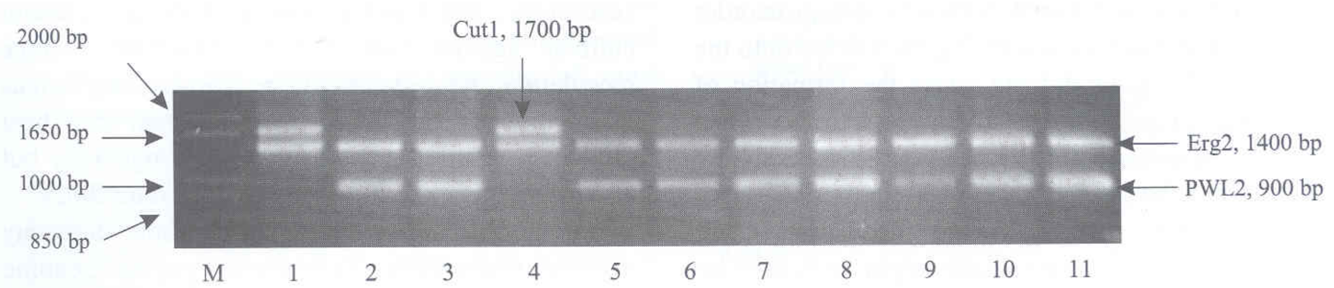

Fig 2 SCAR markers of *P. grisea* were amplified using specific primers. M. marker, 1. isolate *P. grisea* dc4 originated from *D. ciliaris*, 2-4. the derived isolate of *P. grisea* dc4 on Kencana bali (dc4-kb), Cisokan (dc4-c) rice cultivar, and *P. repens* (dc4-pr) grass, 5-6. the derived isolate of *P. grisea* dc4 of Kencana bali on Kencana bali (dc4-kb-kb), Cisokan (dc4-kb-c) rice cultivar, 7-8. the derived isolate of *P. grisea* dc4 of Cisokan on Kencana bali (dc4-c-kb), Cisokan (dc4-c-c) rice cultivar, 9-11. *P. grisea* race 173 originated from rice, and the its derived isolate on Kencana bali (173-kb), Cisokan (173-c) rice cultivar.

P. repens, while dc4-c might not infect it (Table 2). On the other hand, *P. grisea* race 173 was able to infect Kencana bali and Cisokan (Fig 1d and 1e), but was unable to infect IR64, *P. repens, C. dactylon, Digitaria* sp., and *O. nodosa*. The size of lesions on the leaf blade of Kencana bali and Cisokan, due to *P. grisea* dc4 infection, was generally smaller than those of *P. grisea* race 173.

SCAR Diversity. Genome amplification has shown that *P. grisea* dc4 had two out of three SCAR markers, i.e. Cut1 and Erg2, but did not have PWL2 (Fig 2). Their sizes were around 1700 bp and 1400 bp, respectively. Strains derived from inoculated rice (dc4-kb and dc4-c) did not indicate the presence of Cut1 marker, but had PWL2 and Erg2 markers. Their sizes were around 900 bp and 1400 bp, respectively. As *P. grisea* dc4 could not infect cv. IR-64 and the other three grasses, their SCAR genotype could not be explored. No genotype alternation was detected when *P. grisea* dc4 was inoculated to *P. repens*. When *P. grisea* dc4-kb was cross inoculated to *O. sativa* cv. Cisokan or *vice versa*, no changes of SCAR marker could be detected. Genotype alteration was shown neither on *P. grisea* d4-c-kb nor dc4-kb-c.

Unlike *P. grisea* dc4, *P. grisea* race 173 also had two out of three SCAR markers but it had PWL2 instead of Cut1 (Fig 2). The sizes of PWL2 and Erg2 were around 900 bp and 1400 bp, respectively. Inoculation of *P. grisea* race 173 to rice cv. Kencana bali and Cisokan did not change the genotype of SCAR. This race was unable infect to infect IR64, *P. repens, C. dactylon, Digitaria* sp., and *O. nodosa*, so their SCAR genotype could not be explored.

DISCUSSION

Cross-Infection Ability. *Pyricularia grisea* dc4 had a type race of 000 (unpublished) as a result of a physiological race assessment by Balitpa Bogor. The infection method in this experiment was modification of punch infection (Ono *et al.* 2001). Based on the lesion formed, *P. grisea* dc4 was less virulent compared to race 173). Eight days after *P. grisea* dc4 inoculation, lesions on *O. sativa* Kencana bali and Cisokan appeared, but their sizes were smaller compared to the lesions on the same plants that were inoculated with *P. grisea* race 173. Ou (1980) stated that the lesion types are the result of an interaction between resistant rice cultivar and virulent pathogen. Furthermore, Tredway *et al.* (2005) stated that a weak virulent *P. grisea* inoculated to wheat induced limited expansion of lesions, while lesions observed on *Poa pratensis* were 1-2 mm in length 7 d after inoculation with low virulence isolates.

In this study it was also found that *P. grisea* from grass (*D. ciliaris*) might infect cv. Kencana bali and Cisokan. This is not the first report on the ability of *P. grisea* from grass to infect rice. This phenomenon was reported by Singh and Singh (1988) and DBPT (1992). Transmission of *M. grisea* (teleomorph of *P. grisea*) occurs in natural agroecosystems (Kato *et al.* 2000).

Neither *P. grisea* dc4 nor race 173 was able to infect the persistent cv. IR64. *P. grisea* dc4 did not infect its original host as well. Their incapability to infect these plants might be due to decreasing in their pathogenicity during *in vitro* culturing. The same isolate of race 173 did not infect cv. IR64 (Kurnianingsih 2008). Actually, *P. grisea* race 173 was considered as a highly pathogenic race towards all differential rice cultivar series, except to Asahan (DBPT 1992).

There are a lot of inoculation methods. This experiment and also that of Mackill and Bonman (1986) used injection inoculation procedure. According to Guochang *et al.* (1989), inoculation by injection produced higher disease index than spraying. The inoculation using fragmented mycelial spraying onto the 3- to 4-leaf stage of 2 rice cultivars induced the

formation of lesions (Singh and Singh 1988). In this experiment, the inoculation method by syringe in order to put conidial suspension of *P. grisea* dc4-c onto the sheath of *P. repens* did not cause the formation of symptoms. In contrast, a blast lesion was formed when mycelial suspension of *P. grisea* dc4-c was directly injected into the stem of *P. repens* (unpublished data). The symptoms formed in the inoculation using fragmented mycelial suspension might be caused by the fungus that grew directly in the host tissue; this did not need infective structure to penetrate host epidermis. Melanin-deficient *M. grisea* with spraying inoculation failed to infect leaf tissues, but were successfully infected by the wounded leaf (Chumley and Valent 1990). They grow intra- and inter-cellular on susceptible host tissue (Ebbole 2007). In the natural infection, the conidia on the leaf surface will produce germ tubes and swell to form appressorium prior to penetration to host tissue. *Panicum repens* was susceptible to strain from *L. hexandra*, but was resistant to strain from *P. maximum* and *E. crusgalli* (DBPT 1992). *Panicum repens* was also resistant to 3 blast isolates from rice and to 11 isolates from grass species (Mackill and Bonman 1986).

SCAR Diversity. Using SCAR markers, this research may reveal diversity of *P. grisea*. Three SCAR markers used in this study were chosen on the basis of their ability to detect the genotype variation in blast fungus. Prior to this study, those markers were used to indicate haplotype variability of 114 isolates of rice blast pathogen from Lampung as well as 82 isolates from Sukabumi. About 54.4 and 70.7% of those isolates respectively, had all three markers (Reflinur *et al.* 2005). Fourteen SCAR primers that had been used in the study of *P. grisea* population (64 isolates) pathogenic to rice were collected from rice worldwide (Soubabere *et al.* 2000). The Cut1 marker has three alleles with a size range of null, 800-1730 bp (null allele frequency is 0.66). The PWL2 has three alleles with a size range of null, 800-900 bp (null allele frequency is 0.21). The Erg2 has two alleles with a size range of null, 1440 bp (null allele frequency is 0.47) (Soubabere *et al.* 2001).

This study found that host shift might induce diversity in SCAR markers of *P. grisea*. This was shown when *P. grisea* dc4 from grass was transmitted to rice cultivars. However, SCAR markers did not alter when *P. grisea* was transmitted from one rice cultivar to another or from grass to grass. Thus, only host shift from grass to rice induced SCAR genotypic diversity. *Pyricularia grisea* dc4 from grass (*D. ciliaris*) might infect cv. Kencana bali and Cisokan. According to Kurnianingsih (2008), cv. Kencana bali was susceptible, and Cisokan was a moderate resistant cultivar against race 173 by injection syringe inoculation. Re-isolation of rice blast-causing fungus that had been inoculated to a resistant rice host produced variation not only in its pathogenicity but also in its genetic diversity (Namai and Iwade 2002).

The genetic alteration induced by host shift may involve transposon. *Pyricularia grisea* genome containing various transposon has been reported many times. The activity of transposon can alter the genotype. The changes caused by transposon can supply wide genetic variation, especially species that do not have a sexual phase. Stress can stimulate activity of transposable elements (Favaro *et al.* 2005). DNA transposon Pot3 has transposed and provided evidence that its transposon has changed the virulence spectrum of *M. grisea* (Kang *et al.* 2001). Therefore, three SCAR markers used in this study to indicate the genotypic diversity following cross infection need to be re-evaluated by using more samples.

The SCAR marker did not depict its pathogenicity. The cutinase which is encoded by *cut*1 gene of *M. grisea* was neither related to pathogenicity nor affected by sporulation rate (Sweigard *et al.* 1992b) but *cut*2 gene was required for plant infection (Skamnioti and Gurr 2007). The *cut*1 and *cut*2 genes have different DNA sequence. Eight genes of *M. grisea* were putativelly encoded cutinase (Dean *et al.* 2005). The Cut1 of *P. grisea* contains two introns of 115 bp and 147 bp in length and only one copy in its genome. The *cut*1 gene is e xpressed when cutin is the sole carbon source (Sweigard *et al.* 1992a). *PWL*2 is a gene out of four avirulent genes of *M. grisea* strain 70-15 (Dean *et al.* 2005). The existence of *PWL*2 gene in *M. grisea* from rice prevented this fungus from infecting a second grass host, *Eragrostis curvula,* but remained pathogenic to rice and barley. The allele of the *PWL2* gene, *pwl*2-2, was pathogenic to that host, and has been reported as spontaneous pathogenic mutants. The *pwl*2-2 is different from *PWL*2 by a single base pair substitution. With its substitution, *pwl*2-2 had become a nonfunctional. The PWL2 locus is highly polymorphic among blast-causing rice pathogens from diverse geographic locations (Sweigard *et al.* 1995). The presence of PWL2 homologs had no correlation with its pathogenicty on *Eragrostis curvula* (Kang *et al.* 1995). This study also found that the Erg2 marker was a stable locus as this was always detected in the result of cross-inoculation experiments. The *erg*2 gene was stable as this gene is part of an important gene for the cell. This gene in *M. grisea* encodes Δ^8-Δ^7 sterol

isomerase (Keon *et al.* 1994). Sterol is an important component of all eukaryotic cells, and has a role in stabilizing the membrane under stress, and it is related to morphogenetic processes in mycelia of most pathogenic fungi (Mysyakina and Funtikova 2007).

Pyricularia grisea dc4 obtained from *D. ciliaris* had the posibility of a genotype change due to a host shift to rice cv. Kencana bali and Cisokan, but not to *P. repens*. The genotype change was detected in Cut1 and PWL2, but not in Erg2. Once *P grisea* infected rice, the SCAR genotype was apparently stable.

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