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PYRICULARIA ORYZAE: RACES DISTRIBUTION AND SCREENING OF FUNGAL ANTAGONISTS *IN VITRO*

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A B S T R A C T

One of the suggested blast disease management approaches is integrated disease control, which combines cultivation practices, resistant cultivars, and fungicide applications as appropriate. Planting resistant varieties are the most cost-effective option, but the resistance is quickly broken down over seasons and areas due to the existence of many races. Planting resistant varieties should therefore be accompanied by other control methods like biological control techniques. Selecting varieties with resistant gene(s) that match the pathogen race in the field is recommended. It is therefore critical to monitor the race composition in the area. This study aimed to determine races of *Pyricularia oryzae* isolated from different subdistricts in Pinrang, Bone, Maros, and Gowa regencies and the potential of rhizosphere fungal isolates to inhibit the growth of three dominant fungal blast races. *Pyricularia oryzae* was isolated on rice that showed specific symptoms of blast disease. Blast pathogen was grouped into races using 7 rice differential variety sets: Asahan, Cisokan, IR64, Krueng Aceh, Cisadane, Cisanggarung, and Kencana Bali. Observations were carried out 7 days after inoculation based on the standard evaluation system according to IRRI. The antagonists were isolated from the rhizosphere of healthy rice plants, followed by the dual culture test on Potato Dextrose Agar (PDA). A total of 42 isolates were collected from blast-infected rice, and 14 isolates were obtained from the rice rhizosphere. Based on the reactions of 7 differential varieties, a total of 18 races were determined. Races 000 and 001 were dominated with every 6 isolates, followed by races 003 and 020 with 5 isolates each, and races 033 and 013 with 4 and 3 isolates respectively. The other races were found only every two or single isolate. Dual culture using fungal rhizosphere isolates revealed that five isolates had inhibition above 75% against 3 representative races of *P. oryzae.* Based on the findings, it is suggested that the variation of *P. oryzae* races in 4 regencies in South Sulawesi is significantly high, and dominated by 2 races. The five rhizosphere fungal isolates may be used as potential bioagents for the eco-friendly management of rice blast disease.

Keywords: blast disease, biological control agent, *in vitro* screening.

INTRODUCTION

The Central Statistics Agency (BPS) reported that total rice production in Indonesia was 54.42 million tons of milled dry grain (GKG) in 2021. This figure decreased by 233.91 thousand tons or 0.43 percent compared to 2020 rice production, which was 54.65 million tons of milled dry grain (Sopialena and Nurdiana, 2019). One of the factors that affect the lack of rice production is the presence of plant diseases. In Indonesia, blast disease caused by *Pyricularia aryzae* was ranked fourth in causing harvest failure during 2015-2020. The distribution area of blast disease in Indonesia in the 2020 planting season reached 22.620 ha and is expected to increase to 32.391 ha in the 2021 planting season. This disease initially attacked upland rice, but nowadays already spread to paddy fields and even to swampy rice fields (Asibi *et al*., 2019). The area of disease attacks during the last 10 years has reached 9.778 ha/year. The severity of the disease can reach 90% of neck blast attacks with yield losses of 50-90% on a susceptible variety. This is because *P. oryzae* can infect rice plants from nursery to grain filling. Symptoms caused by the attack of the fungus *P. oryzae* are in the form of spots on leaves, stem books, panicle necks, and leaf midribs (Bushal, 2021; Cruz and Valent, 2017). In the vegetative phase, there are

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rhombic spots on the leaves, and in the generative phase, there are neck rot spots. Serious attacks in the vegetative phase can cause the death of rice plants whereas in the generative phase can cause panicle neck fractures and empty rice grains (Bastiaans, 1991; Shahriar *et al*., 2020).

Pyricularia oryzae has different virulence which can be determined based on the response of 7 standard varieties. The distribution of *P. oryzae* races of varies in each region. Race identification of *P. oryzae* in Papua Barat reported by Santoso *et al*. (2019) revealed 9 race groups namely race 211, 213, 241, 251, 253, 313, 333, 353 and race 373, which were dominated by race 333 as much as 41.18% and race 373 by 33.35%. The *P. oryzae* races obtained are races that have a high virulence level. The presence of 17 different races between 30 *P. oryzae* isolates in Maros, however, the dominance race was 001 that has low virulence in comparison with *P. oryzae* isolates in Papua.

Blast disease can be controlled through a variety of methods, including cultivation techniques, the planting of resistant varieties, and the application of fungicides. However the use of resistant varieties is the most effective, economical, and simple; however, this technology is challenged by *P. oryzae*, which has high genetic diversity, adaptability, and the ability to form new races capable of breaking the resistance of newly introduced varieties. These new varieties can only grow for two to three seasons (Syarif *et al*., 2017; Longya *et al*., 2020; Simkhada and Thapa, 2022; Sudir *et al*., 2014; Wang *et al*., 2013). The ability to carry out recombination both sexually and asexually is the cause of this dynamic population formation (Naor *et al*., 2012). Using synthetic fungicides in addition to endangering human health also negatively impacts agricultural land and causes agricultural products to be unsafe for consumption. The use of resistant varieties is an effort to control blast disease which until now is considered the most effective assisted by the use of other control components such as fungicides. However, it is difficult to control this disease because *P. oryzae* has high genetic diversity and highly adaptive cellular and morphological developmental traits in infected rice plants. These traits cause the fungal races of *P. oryzae* to change their virulence properties in a short time, depending on the host and changing climatic conditions (Khemmuk, 2017). Alternative disease control includes the use of microbial antagonists applied to seeds before planting. The antagonists can exhibit several direct or indirect mechanisms of action involved in biological disease control. These mechanisms include; antibiosis, mycoparasitism, induced resistance, and growth enhancement. Secretion of extracellular hydrolytic enzymes by the antagonist, competition for space and nutrients between organisms and detoxification of virulence factors are other actions involved in biological disease control (Deketelaere *et al*., 2017; Heydari and Pessarakli, 2010; Junior *et al*., 1990; Zhang *et al*., 2014). Application of the bio-control agents i.e. *Trichoderma viridae* or *Pseudomonas fluorescens* and the use of resistant cultivars are the sustainable and eco-friendly approach of blast control and other major pathogen on rice (Hajano *et al*., 2012; Kabdwal *et al*., 2023; Yadav, 2018). The biocontrol approach for managing blast disease was considered a good alternative. Fungal antagonists play a significant role in controlling plant pathogens and diseases and they are used as biocontrol agents throughout the world. In some cases, the symbiosis of microorganisms with plant roots has a large impact on the fulfillment of plant nutrition and on controlling plant pests and diseases. Many fungal species ie. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Curvularia* sp., and *Trichoderma harzianum* from the rhizosphere were reported to be potential antagonists against blast pathogen *Pyricularia oryzae*. Based on these facts, research was conducted on the isolation and characterization of both fungal isolates from rice rhizosphere and blast fungus from infected rice, determination of *P. oryzae* races on a standard differential varieties as well as testing of the effectiveness of the fungal rhizosphere against three different races of *Pyricularia oryzae in vitro*.

MATERIALs AND METHODS

Collection of *Pyricularia oryzae* Isolates: A sampling of infected plants was carried out using the purposive sampling method on vegetative and generative phases of rice plants. Spore drop method modified from a method was used. Each rice blast lesion on an infected leaf was cut in half, with a healthy section on one end of each half. Surface sterilization was performed with 1 % solution of commercial bleach (Clorox®) containing sodium hypochlorite for 1 minute, followed by three rinses with sterilized distilled water to remove remains of Clorox.. Each lesion fragment was then adhered to the upper-lid of a Petridish containing 2% water agar, with the adaxial part facing the medium. The plates were then placed in a humidity box at room temperature and examined daily with a light microscope for a single spore colony of *P. oryzae*. Each spore colony was then selected and transferred to oatmeal agar (OMA; 15 g of instant oatmeal).

Characterization and Determination of *Pyricularia oryzae* races: Spore suspension samples suspected of containing blast pathogens were identified by shape and

type and examined under a light microscope at various magnifications (Bonman *et al*., 1986). The presence of conidia that are club-shaped in gray and have two septa, but sometimes one or three septa, distinguishes the Po fungus. Isolates containing Po fungi were stored as pure stock in PDA agar or 10% glycerol.

Determination of *P. oryzae* race according to the method of Balai Besar Padi, Sukamandi modified by Prabawa and Damanhuri (2018), using 1 set of differential varieties consisting of 7 varieties: Asahan, Cisokan, IR 64, Krueng Aceh, Cisadane, Cisanggarung, and Kencana Bali. The soil preparation followed the method from Lestari (2016), while the preparation of *P. oryzae* inoculum. The intensity of blast attacks was observed seven days after inoculation using the IRRI evaluation standard (International Rice Research Institute, 1996).

Isolation of Fungal Rizosphere: Soil samples were collected from a healthy rice plantation in Moncongloe, Maros regency to obtain several fungal isolates having the antagonistic potential against different *Pyricularia oryzae* races. The soil dilution plate method was used to collect isolates. Fungal isolates were then evaluated for their antagonistic potential against *P. oryzae* races. The antagonist activity was assessed by dual culture assay on potato dextrose agar (PDA).

In vitro* evaluation of the antifungal activity of soil fungi against *P. oryzae: *In vitro* antagonist test was performed on all fungi isolated from the rhizosphere of rice plants, according to (Sharma and Gupta, 2014). Diagonal lines were made on the outer surface of the Petri dish containing Oatmeal agar (OMA) media. Tests were carried out by growing fungal isolate colonies in pairs. Pure cultures of *P. oryzae* and each fungal isolate were inoculated in a petri dish containing OMA media. *Pyricularia oryzae* was grown onto OMA media unpaired as control. The distance between the rhizosphere fungus and *P. oryzae* was 3 cm and 3 cm from the edge of the petri dish. Each treatment was repeated three times. After the incubation period, the inhibition of the growth radial of each fungus was measured and calculated using the formula below:

$$\text{IGR} = \frac{\text{d1} - \text{d2}}{\text{d1}} \times 100\%$$

where:

IGR = Inhibition Growth Radial (%)

d1 = Radial growth of control (mm)

d2 = Radial growth of treatment (mm)

Morphological characterization of fungal isolates: The fungal determination key developed by Barnett and Hunter was used to describe fungal isolates from rice rhizosphere used more recent ones to define the genres to which they belong.

DATA ANALYSIS

The data obtained were analyzed statistically by means of variance, then if significantly different data were found, it would be continued with the Duncan Multiple Range Test (DMRT) at the 5% level.

RESULTS AND DISCUSSION

Isolation and Characterization of Blast Pathogen : A total of 20 field isolates of *P. oryzae* were recovered from symptomatic rice leafs, necks, and panicles in 2021-2022 growing seasons in 5 sub districts of Pinrang, and 22 isolates from 8 sub districts in Maros, and Gowa regencies from 6 rice cultivars (Table.1).

Table 1. Origin of Rice blast isolates

No	Isolates Code	District/Sub District	Plant Tissue	Rice Variety
1	PoP1-PoP6	Pinrang/Paleteang	Leaf	Inpari 8
2	PoP7-PoP8	Pinrang/Tiroang	Leaf	Inpari 8
3	PoP9-PoP12	Pinrang/Watang Sawitto	Leaf	Inpari 9
4	PoP13-PoP17	Pinrang/Mattirobulu	Leaf	Inpari 8
5	PoP18-PoP22	Pinrang/Suppa	Leaf	Inpari 8
6	PoB1-PoB8	Bone/Bengo	Leaf	Inpari4
7	PoM1-PoM8	Maros/Simbang	Neck	Ciherang
8	PoG1-PoG4	Gowa/Patallassang	Collar	Ciliwung

The morphological characteristics of 42 rice blast isolates were examined. Fungal colony color on potato dextrose agar (PDA) and on oat meal agar (OMA) plates were observed, and each isolates showed different characteristics in terms of appearance and color. The presence of gray conidia and mycelia was observed in the macroscopic characteristics of the sporulating *Pyricularia oryzae* on incubated leaf. Conidiophores are single and range in color from gray to hyaline, conidia are formed at the tips of conidiophores. On potato dextrose agar (PDA)

media, *P. oryzae* isolates displayed variations of colony color, ranging from white and blackish gray, or brown. The rear colony is black, blackish gray, or a combination of brown and black in color. Colony patterns are typically circular, resembling concentric rings that lead to a growing center point. Some isolates had smoother colony margins, while others had irregular ones. The colony generally has a cotton-like texture (Figure 1 A, B, and C). *Pyricularia oryzae* colonies grown on OMA media were more uniform, with black-gray in front and black or dark brown in back. The texture of the colonies is also more uniform, i.e. velvety, and the colony patterns are generally circular in shape, resembling concentric rings that lead to a growing center point (Figure 2 D, E and F). After 7 days of culture incubation, a dark melanin pigment was observed on the fungal colony. The conidia shape was observed under a microscope and generally had a pyriform shape with two to three septate (Figure 1 G and H).

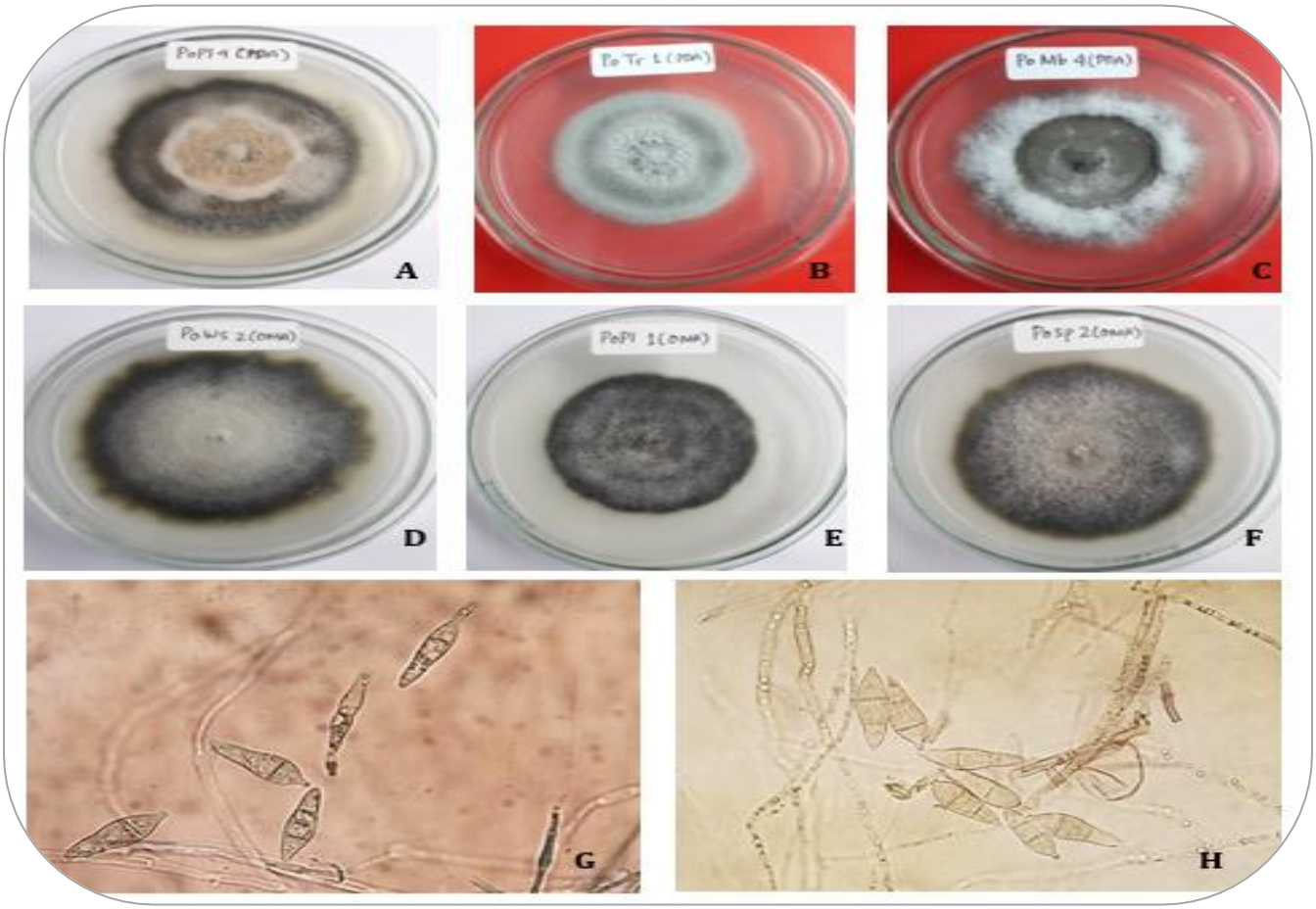


Figure 1. Variation in colony color, colony pattern, and the form of conidia among rice blast isolates on potato dextrose agar (PDA) and on oat meal agar (OMA), 7 days after incubation.

Determination of *Pyricularia oryzae* Races: The races of *P. oryzae* were determined using seven different varieties' responses. Within 42 isolates, 16 races were discovered. Races 020 (8 isolates), 003, and 111 (6 isolates each) were dominated and distributed in the regencies of Pinrang, Bone, Maros, and Gowa. The other races were determined using only a few isolates. Pinrang isolates contained a total of ten races, whereas Bone, Maros, and Gowa isolates contained seven, six, and three races, respectively (Table 2). Race 001 can only infect the Kencana bali variety, while race 003 can attack the Cisanggarung, and Kencana Bali varieties. The Krueng Aceh variety was sensitive to race 020, whereas the other six varieties were resistant to race 020. Five other varieties were resistant to race 003, whereas the Cisanggarung and Kencana Bali cultivars were susceptible. There are three susceptible rice varieties for race 111, namely Cisokan, Ciherang and Kencana Bali. The results of the analysis revealed that Asahan and IR-64 had the broadest spectrum of resistance. The Asahan variety showed a susceptible response only to race 111, whereas the IR 64 strain was susceptible to *P. oryzae* races 041, 073, 173, and 251 respectively.

Table 2. Reactions of 7 Rice Differential Varieties to 42 different *Pyricularia oryzae* isolates

Isolate	Races	Reaction of rice differential varieties						
		Asahan	Cisokan	IR64	Krueng Aceh	Ciherang	Cisanggarung	Kencana Bali
PoP1	020	R	R	R	S	R	R	R
PoP2	020	R	R	R	S	R	R	R
PoP3	023	R	R	R	S	R	S	S
PoP4	003	R	R	R	R	R	S	S
PoP5	033	R	R	R	S	S	S	S
PoP6	033	R	R	R	S	S	S	S
PoP7	020	R	R	R	S	R	R	R
PoP8	003	R	R	R	R	R	S	S
PoP9	001	R	R	R	R	R	R	S
PoP10	111	R	S	R	R	S	R	S
PoP11	003	R	R	R	R	R	S	S
PoP12	111	R	S	R	R	S	R	S
PoP13	020	R	R	R	S	R	R	R
PoP14	001	R	R	R	R	R	R	S
PoP15	111	R	S	R	R	S	R	S
PoP16	003	R	R	R	R	R	S	S
PoP17	013	R	R	R	R	S	S	S
PoP18	013	R	R	R	R	S	S	S
PoP19	041	R	R	S	R	R	R	S
PoP20	033	R	R	R	S	S	S	S
PoP21	101	R	S	R	R	R	R	S
PoP22	073	R	R	S	S	S	S	S
PoB1	111	R	S	R	R	S	R	S
PoB2	000	R	R	R	R	R	R	R
PoB3	001	R	R	R	R	R	R	S
PoB4	003	R	R	R	R	R	S	S
PoB5	003	R	R	R	R	R	S	S
PoB6	020	R	R	R	S	R	R	R
PoB7	023	R	R	R	S	R	R	S
PoB8	102	R	S	R	R	R	S	R
PoM1	173	R	S	S	S	S	S	S
PoM2	000	R	R	R	R	R	R	R
PoM3	020	R	R	R	S	R	R	R
PoM4	101	R	S	R	R	R	R	S
PoM5	251	S	R	S	R	S	R	S
PoM6	111	R	S	R	R	S	R	S
PoM7	000	R	R	R	R	R	R	R
PoM8	111	R	S	R	R	S	R	S
PoG1	010	R	R	R	R	S	R	R
PoG2	020	R	R	R	S	R	R	R
PoG3	100	R	R	R	R	R	R	R
PoG4	020	R	R	R	S	R	R	R

Note: R : Resistant S : Susceptible

Dual Culture of Rhizosphere Isolates against *Pyricularia oryzae:* A total of 14 isolates were isolated from the rice rhizosphere in Moncongloe District, Maros with different characteristics of color, texture, and colony margins. The percentage of inhibitory radial growth of the 14 isolates was significantly different during observations. The development of radial growth inhibition of *P. oryzae* race 020, 003 as well as 111 on dual

culture after 8, and 12 days of observation can be seen in Table 3. At 8 dpi, isolates MR6, MR7, MR13 and MR14 had the highest percentage of growth inhibition toward *P. oryzae* race 020, while toward race 11 were observed on isolates MR6, MR11, MR13 and MR14 respectively. Similar result was obtained against race 033. The highest radial growth inhibition was showed by isolates MR1, MR4, MR6 and MR14. On 12 dpi, isolates MR6, MR7, MR13 and MR14 showed an inhibition percentage of 100% toward race 020, and significantly different from other tested isolates. The best growth inhibition of *P. oryzae* races 111 and 033 were also obtained by isolates MR6, MR11, MR13 and MR14, and was significantly different in compare to other isolates. The lowest growth inhibition of all three *P. oryzae* races was observed on isolates MR3 and MR9.

Tabel 3. Percentage of Inhibitory Radial Growth of *Pyricularia oryzae* Race 020, Race 111 and Race 033 in dual culture test, 8 and 12 days post inoculation.

Isolates	Treatments					
	Race 020		Race 111		Race 033	
	8	12	8	12	8	12
MR1	34,67^{cd}	65,57^{b}	65,53^{b}	78,74^{bc}	77,67^{ab}	89,67^{abc}
MR2	55,33^{b}	60,45^{bc}	47,12^{c}	64,83^{cd}	51,50^{cde}	66,90^{cd}
MR3	17,50^{def}	32,59^{de}	26,99^{de}	40,97^{ef}	8,67^{fg}	29,11^{f}
MR4	50,00^{bc}	66,42^{b}	49,14^{c}	63,04^{cd}	84,67^{ab}	72,86^{bc}
MR5	17,33^{def}	19,15^{ef}	18,21^{ef}	23,91^{fg}	36,67^{ef}	41,78^{ef}
MR6	100,00^{a}	100,00^{a}	100,00^{a}	100,00^{a}	100,00^{a}	100,00^{a}
MR7	87,07^{a}	100,00^{a}	41,67^{cd}	41,19^{ef}	45,76^{def}	44,64^{def}
MR8	20,67^{de}	47,70^{cd}	27,78^{de}	57,62^{de}	21,45^{efg}	43,81^{def}
MR9	13,40^{ef}	17,04^{f}	4,74^{fg}	15,78^{gh}	6,56^{g}	20,05^{fg}
MR10	49,36^{bc}	66,67^{b}	40,69^{cd}	55,63^{de}	45,04^{def}	66,26^{cde}
MR11	63,46^{b}	71,14^{b}	77,12^{b}	90,29^{ab}	67,38^{cd}	91,89^{ab}
MR12	24,68^{de}	35,70^{d}	22,71^{e}	32,56^{fg}	18,97^{efg}	42,57^{def}
MR13	92,31^{a}	100,00^{a}	100,00^{a}	100,00^{a}	73,76^{bc}	100,00^{a}
MR14	100,00^{a}	100,00^{a}	100,00^{a}	100,00^{a}	100,00^{a}	100,00^{a}
KONTROL	0,00^{f}	0,00^{g}	0,00^{g}	0,00^{h}	0,00^{g}	0,00^{g}

Ket : Numbers followed by the same letter in the same column show no significant difference in Duncan's Multiple Test at 5% level

Antagonistic Mechanism: The isolates of antagonistic fungi showed an interaction mechanism in the form of competition. What is intriguing is the presence of antibiosis interactions observed in *T. harzianum* testing. *Trichoderma* was seen growing rapidly at the culture medium's edges and covering the *P. oryzae* colonies, but there was a clear zone in the middle that was not overgrown by *P. oryzae*. (Figure 2c). In addition to competition, in *Penicillium* 1 and *Penicillium* 2 isolates, hyperparasitic interactions were observed at the meeting point of both colonies and the *P. oryzae* (Figure 2d and 2e). Macroscopically, the inhibition mechanism of the rhizosphere antagonist isolates on the growth of *P. oryzae* began to appear at 6 dpi. The majority of rhizosphere fungal isolates developed more quickly than *P. oryzae* colonies. *Trichoderma*, *Aspergillus*1, and all of them quickly covered practically the entire surface of the media.

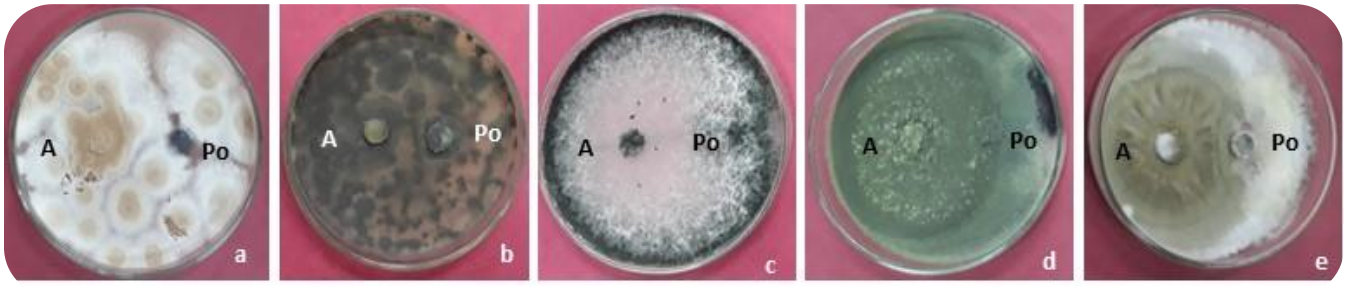
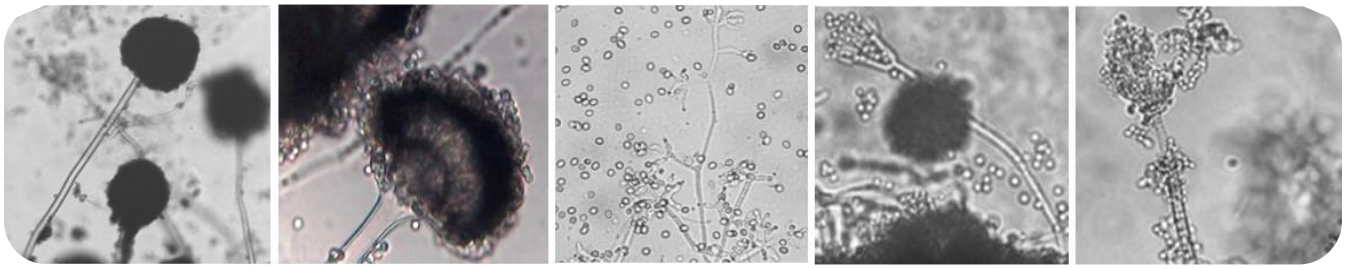


Figure 2. *In vitro* antagonism test between rhizosphere fungal isolates and *P. oryzae* on 12 *dpi*; (a) *Aspergillus* sp1; (b*) Aspergillus* sp2; (c) *Trichoderma harzianum*.; (d) *Penicillium* sp1.; and (e) isolates of *Penicillium* sp2.

Morphological Identification of Antagonist Fungi: The five rhizosphere isolates with highest percentage of *P. oryzae* races 020, 003, and 111 radial growth inhibitions were macroscopic and microscopically identified. Further macro- and microscopical observations confirmed these findings. Color, texture, and the colony's edge are all visible under a microscope, whereas under a microscope, the septa of hyphae, color, the shape of conidia or spores, and the morphology of conidiophores are all visible. Based on the macroscopic and microscopic characteristics, isolates were grouped into the genus *Penicillium*, *Aspergillus*, and *Trichoderma*. Isolates MR1 and MR6 were identified as *Penicillium,* isolates MR11 and MR14 were identified as *Aspergillus*, and MR13 isolate as *Trichoderma*. Conidiophores of MR11 and MR14 end in an apical vesicle, and at the other end, a basal foot cell is inserted into the supporting hyphae. Phialides are chains of conidia that are either directly linked to the vesicle or to a cell called a metulae in between (Figure 3). For further molecular identification, all of these isolates will analyze by using two universal primers internal transcribed spacer ITS1 and ITS2, followed by sequencing.



A B C D E

Figure 3. Microscopic feature of five rice rhizosphere isolates potential as biocontrol agents of P. oryzae races 020, 222 and 003. *Aspergillus* sp1 (a), *Aspergillus* sp2 (b), *Trichoderma* (c), *Penicillium* sp1(d), *Penicillium* sp2 (e).

DISCUSSION

Symptoms of blast attack resulting from inoculation using seven differential varieties showed that in resistant varieties only small brown spots appeared and did not experience significant development, while in susceptible varieties a distinctive rhombus-shaped spot appeared with a gray to dark-gray sporulation center. white. TeBeest *et al.* (2007), said that the symptoms of blast attack on leaves generally appear rhombus-shaped spots. Symptoms on leaves vary according to environmental conditions, age of the plant and the level of resistance of the host cultivar. On sensitive plants, the initial spots appear gray surrounded by dark green color and then grow to several centimeters while the spots on resistant plants often remain 1-2 mm in size and are brown to dark brown.

Yuliani and Maryana (2014) reported that the dominance of the blast pathogen race in one region with another, allows rice varieties in one area to be resistant but susceptible in another, because these pathogenic races have different virulence based on the place and growing season. By knowing the distribution of the dominant *P. oryzae* race in a blast endemic area, the control of blast disease will be more effective by using resistant varieties adapted to the *P. oryzae* race in that area. According to Mogi *et al*. (1992), Asahan is the variety with the highest resistance to blast disease, while the Kencana bali variety is always used as a susceptible check plant in blast disease testing in Indonesia (Mukelar and Edwina, 1987). The symptoms of blast disease arise due to compatible reactions between plant resistance genes (R genes) and pathogenic avirulent genes (avr genes), as stated by Agrios (2005) in (Lestari, 2016).

Of all 14 potential fungal isolates isolated from the rhizosphere of healthy rice plants, can be differentiated based on the color, texture, and surface of the colony. These changes in features demonstrate the diversity of fungal species that interact with one another in the plant rhizosphere. This supports Pandit *et al*. (2020) claim that the rhizosphere is a zone where interactions between plants, soil, and micro-organisms close to the roots take place. The majority of plant-associated microorganisms are found and active in the rhizosphere. In the rhizosphere, pathogenic soil bacteria, living things and other microbes interact and compete for nutrients.

Tests for antagonistic activity against P. oryzae races 020, 003, and 111 were performed on all 14 isolates. The *P. oryzae* isolates tested were the dominant races found on rice plantations in three districts in South Sulawesi, namely race 020 in Gowa Regency, race 003 in Bone Regency, and race 111 in Maros Regency. The development of inhibition of MR14, MR13, MR6, MR7, and

MR11 isolates on the growth of *P. oryzae* race 020 showed the highest inhibitory effect at 12 dpi compared to other isolates. The same result was also found against *P. oryzae* race 003. Isolates MR14, MR6, MR13, MR11, and MR1 had the highest percentage of inhibition at 12 dpi which was above 90%. The same thing happened to the development of the percentage of inhibitory power of isolates MR14, MR13, MR6, MR11, and MR1 also ranked fifth highest in suppressing the growth of *P. oryzae* race 111 at 12 dpi, although isolates MR11 and MR1 at 4 dpi had inhibitory percentages below 50 %. The difference in the percentage of inhibition of each isolate against *P. oryzae* in three types of races proves that races of *P. oryzae* have different pathogenic and virulence properties. This agrees with Kariaga *et al*. (2016) who state that the population of *P. oryzae* is very diverse and consists of individual races that have different virulence properties. Pathogenicity is influenced by differences in metabolic mechanisms and chemical compounds found in the fungus *P. oryzae* (Norvienyeku *et al.,* 2021).

The analysis of variance shows that the interaction between the rhizosphere fungus and the P. oryzae race is significant. The rhizosphere fungus (Factor A) gave a significant effect, while the races of *P. oryzae* (Factor B) gave no significant effect in growth inhibition. Further analysis using Duncan's Multiple Test at the 5% level, showed that isolates MR6, MR13, and MR14 had a 100% inhibition but were not significantly different from isolates MR1 and MR11 (data not shown). This result is not much different if compare with other race of *P. oryzae.* The fungus *P. oryzae* is a plant pathogen that has variable virulence properties so that it can easily form new races. This is in accordance with the opinion of which stated that isolates of rice blast fungus tend to be unstable in colony appearance, fertility, and pathogenicity during repeated subcultures in the laboratory.

Identification of rhizosphere isolates was carried out on isolates that had the highest percentage of inhibition, including isolates MR1, MR6, MR11, MR13, and MR14. The results of macroscopic and microscopic identification of MR1 isolates showed that the isolates were *Penicillium* sp1. as seen from the branched conidiophores and round conidia. This agrees with Barnett and Hunter (1998) who stated that the conidia form of *Penicillium* sp. spherical or ovoid, conidiophores branched, phialide ends produce conidia. Macroscopically, the MR6 isolate has a dark green color with yellow spots and microscopically has branched conidiophores, phialide ends produce conidia that are round. From these characteristics, isolate MR6 referred to as *Penicillium* sp2. This agrees with Arné & Lee, (2019) who said that macroscopically the texture of *Penicillium* sp. is smooth velvety to powdery, green, blue-green, and gray-green in color, often with white edges. Sometimes exudates of various colors may also appear on the surface and on the back a pale cream or yellow color. Microscopically Visagie *et al*. (2021) states that an important feature of Penicillium is the branching shape of its conidiophores. Phialide is the tip of the conidiophores that will produce conidium. The macroscopic and microscopic identification of MR11 isolates showed the color of the colonies was light brown to dark brown and had long conidiophores with rounded ends, that refer to *Aspergillus* sp1. MR14 isolates had a dark green colony and microscopically it has short conidiophores with subclavate vesicles, and conidia in round or semi-round shapes arranged in chains so that from the characteristics of the colonies and microscopically, MR14 isolates resembled the fungus *Aspergillus* sp. Meiniwati *et al*. (2014) said that the conidia of A. fumigatus were green with short, smooth, and transparent conidiophores. Microscopically *Aspergillus* sp. has round or semi-spherical conidia, metulae, and vesicles. On the other hand, MR13 isolates were green and white on the upper surface colonies, conidiophores branched perpendicularly, and phialides at the ends produced round conidia. Based on these characteristics, the MR14 isolate is similar to the fungus *Trichoderma*. This agrees with Barnett and Hunter (1998) who stated that the conidiophores of the fungus *Trichoderma* were hyaline in color, branched, single phialide on each branch, and conidia were round and had fast growth.

Five isolates were identified as antagonistic fungi because they could inhibit the growth of *P. oryzae* race 020, 003, and 111. The inhibition mechanism of the five isolates was different. The isolates of *Penicillium* sp. namely MR1 and MR11 showed inhibition and at 12 dpi the *Penicillium* isolates covered P. oryzae colonies, which was indicated by the presence of yellow color at the meeting of the two colonies as shown in Figure 10e. This indicates that the antagonist fungi produce antibiotic compounds. According to Hidayah *et al.* (2022), the mechanism of antibiosis in the antagonist test is marked by the presence of yellow color at the meeting of the mycelium with pathogenic fungi. *Penicillium* sp. produces an antibiotic compound, namely Penicillin. Penicillin has the ability to inhibit cell wall synthesis (Putra and Purwantisari, 2018).

The results of the antagonist test of *Aspergillus* sp isolates in suppressing *P. oryzae* at 6 dpi to 16 dpi showed inhibitory properties of parasitism and competition as shown in Figure 5. At 12 dpi the percentage of inhibition was 100%. This agrees with Melo *et al*. (2006) who used the fungus *Aspergillus* sp isolated from soil to suppress the pathogen *Sclerotinia sclerotiorum*, the results obtained were that *Aspergillus* was hyperparacytic and caused 100% death of *S. sclerotia.*

In order to manage plant-pathogenic organisms, microbial biocontrol agents (BCAs) are often fungal or bacterial strains isolated from the phyllosphere, endosphere, or rhizosphere. Infection of the host plant by the pathogen or establishment of the pathogen there is prevented by biocontrol agents or microbial antagonists. It has been considered that the main control mechanisms are those that focus primarily on pathogens. The antagonists may display a variety of direct or indirect biological disease control strategies. These include induced resistance (inducing a plant's defensive mechanism against an antagonist), mycoparasitism (where the antagonist obtains some or all of its nutrition from the fungal host), and antibiosis (where the antagonist produces an inhibitory metabolite or antibiotic), induced resistance (the process of inducing a plant's defense response against a plant pathogen), mycoparasitism (when the antagonist obtains some or all of its nutrients from the fungal host), and growth promotion (BCAs promote plant growth while the effects of the disease are being reduced and also through microbial hormones such as indoleacetic acid and gibberellic acid). Other actions involved in biological disease management include the antagonist's secretion of extracellular hydrolytic enzymes, competition between organisms for nutrition and space, and detoxification of virulence agents (Deketelaere *et al.,* 2017; Dong *et al.,* 2016; Heydari and Pessarakli, 2010; Junior *et al.,* 1990; Zhang *et al.,* 2014).

The mechanism of inhibition by *Aspergillus* sp. isolates in suppressing the growth of *P. oryzae* is competition. The fungi *A. flavus, A. fumigatus, A. niger,* and *Curvularia* sp. inhibit by competing, lysing the cell wall of pathogens, and producing antibiotic substances called the antibiosis mechanism. Putri *et al*. (2022) revealed that *Aspergillus* sp. produces glucanase enzymes that function to degrade cellulose in *P. grisea* fungi and inhibit the growth of these pathogenic fungi. *Aspergillus* sp. also produces ochratoxin A, gliotoxin, verrucollagen, fumitremorgin, and trip equivalent. The results of the antagonist test of *Trichoderma* sp. on the growth of P. oryzae showed 100% inhibition by means of competition and parasitism. *Trichoderma* sp isolates had a very fast growth ability so they were able to control space and nutrients in artificial media compared to *P. oryzae* which had relatively slow growth. This is supported by the statement of Meiniwati *et al*. (2014) that fast-growing fungi are superior in controlling space and nutrients so that they can suppress the growth of their opponent fungi. Agrios (2005) stated that the most common mycoparasite fungus is *Trichoderma,* especially *T. harzianum* which has been found to parasitize *Fusarium, Rhizoctonia*, *Sclerotium,* and *Pythium.*

CONCLUSION

There were 16 detected races among the 42 isolates. Races 020 (8 isolates), races 003, and 111 (6 isolates each) dominate and are widely distributed throughout Pinrang, Bone, Maros, and Gowa Regencies. Only a few of the isolates tested belonged to the other races.

There were a total of 14 fungal isolates obtained from the rhizosphere of rice plants. *Penicillium* MR1, Penicillium MR6, Aspergillus sp1 (MR11), Aspergillus sp2 MR14, and Trichoderma sp (MR13) were the best antagonist isolates with a percentage of inhibition above 75% in suppressing the growth of P. oryzae race 020, 003 and 111. The mechanism of inhibition of the five types of antagonist fungi was competition, parasitism, and/or antibiosis.

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Baharuddin Patandjengi	:	Designing of field observation, compilation and interpretation of data
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