First report of *Pyricularia oryzae,* the cause of blast disease in upland rice, in Lombok, West Nusa Tenggara, Indonesia

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Abstract. *Sanuriza II, Suprapta DN, Kawuri R, Suriani NL, Sudantha IM, Jayadi I, Ihwan K. 2024. First report of* Pyricularia oryzae, *the cause of blast disease in upland rice, in Lombok, West Nusa Tenggara, Indonesia. Biodiversitas 25: 683-689.* Rice (*Oryza sativa* L.) is a main crop cultivated by farmers in Indonesia. The demand for rice has increased, but rice production has decreased due to attacks by plant pests, including pathogen blast disease. This research aims to (i) describe the symptoms of blast disease in upland rice in Lombok, (ii) determine the incidence of blast disease in upland rice in Lombok, and (iii) identify the pathogen that causes blast disease in upland rice in Lombok. The research was conducted in Lombok, West Nusa Tenggara. Samples were taken randomly from the dryland area cultivating rice crops, which found 50 locations potentially attacked by blast disease. At one location, three samples of rice crops with blast symptoms were randomized by walking zigzag, resulting in 150 samples of rice crops. Samples were labeled and stored in plastic bag sampling, isolated, and identified at the laboratory. The study results showed that the macroscopic characteristics of rice leaves affected by blast disease could be seen from brown spots and points like a rhombus; the edges are brown and grey or whitish in the center of the spots, and the pathogen-produced white mycelium with a fine structure. In contrast, microscopic characteristics showed that the shape of the hyphae was septate, the structure of conidiophore with autophagic cell death, long pyriform microconidia, and long pyriform septate macroconidia. Based on 18S rRNA gene analysis, the Blast Lombok isolate had a DNA fragment of ±550bp. Based on these results, it can be concluded that the cause of blast disease in upland rice in Lombok, West Nusa Tenggara, is *Pyricularia oryzae*.

Keywords: Blast disease, Lombok, *Pyricularia oryzae*, upland rice, West Nusa Tenggara

INTRODUCTION

Rice (*Oryza sativa* L.) is a main crop cultivated by farmers in Indonesia; the demand for rice increases every year, along with the increase in population. With an interval of 5 years from 2015 to 2045, the average population growth is projected to reach 9.564 million people/5 years, bringing the population in 2045 to 330.90 million people (United Nations 2019). Meanwhile, the average national rice consumption in 2005-2017 was 89.05 kg/capita/year, 96.33 kg/capita/year, and 97.05 kg/capita/year in 2019, continuing to increase on average to 3.39 kg/capita/year, making Indonesia the world's largest rice consumer per capita (Octania 2021). The increasing rice consumption rate is not followed by national rice production capacity. National rice production decreased by 4.60 million tonnes (7.76%), from 59.20 million tonnes of Milled Dry Grain (MDG) in 2018 to 54.60 million tonnes of MDG in 2019 (FAO 2023).

The decline in rice production also occurred in West Nusa Tenggara (NTB). In 2018, rice production in NTB reached 1.46 million tons of MDG, but in 2019, it fell to 1.4 million tons of MDG, the equivalent of 4.11%. Furthermore, the average NTB rice productivity in 2018 was 50.49 ku/ha, then decreased in 2019 to 49.78 ku/ha, or the equivalent of 1.41% (Statistic Indonesia 2020).

According to Ritung et al. (2015) and Jamal et al. (2023), two main problems to declining rice production and productivity include converting productive agricultural land to non-agricultural land and high incidents of plant pest attacks. Strategic actions are needed to solve this problem, such as increasing the area of agricultural land by utilizing dryland.

Dryland is a land resource with great food production potential (Antriyandarti 2023), and NTB is one of the provinces with large dryland potential. NTB has dryland covering an area of 15.9 million ha, or 92.7% of its total land area, spread across the lowlands and highlands (Ritung et al. 2015). That dryland development can be optimized to increase agricultural land for food crops, especially rice.

Limited water availability and blast disease attacks hamper the development of rice crops in drylands. This disease can reduce the quality and quantity of rice crops when water availability is limited in field areas. Blast disease can damage rice crops at all stages, from the seedling vegetative to reproductive phases (Chung et al.

2022). Increasing rice production through agricultural extensification and protecting crops from pest attacks must be done to solve those problems. Therefore, controlling pest attacks means keeping the quality and quantity of rice yields.

Blast disease is one of the pests causing Indonesia's rice production decline (Kuswinanti et al. 2023). A high-intensity attack by blast disease on rice crops resulted in farmers' losses (Nalley et al. 2016; Simkhada and Thapa 2022). The risk of decreasing rice yields does not only occur in Indonesia but also several other Asia countries such as Japan, India, Korea, China, Philippines, and Vietnam have experienced losses of 60%, 7.5%, 8%, 14%, 67%, and 60%, respectively, then in Europe, such as Italy at 24%, and even in America such as Brazil, there have been losses of up to 100% (Wang et al. 2014; Asibi et al. 2019).

Blast disease potentially attacks every part of rice organs in the fields (Muimba-Kankolongo 2018). Kuswinanti et al. (2023) reported the highest percentage of blast disease attacks in Indonesia found on rice leaves, starting from 5% in the first week and reaching 82.1% at the harvest period. Potential yield losses due to blast disease can reach 50% or more, as reported by Nalley et al. (2016), following the next survey in 2021, a field survey conducted in two districts on Lombok Island, including Central Lombok Regency, attacked by blast disease at 70% and North Lombok Regency at 20%. Based on the information described above, it is necessary to identify the pathogenic causes of blast disease to be controlled so rice crops can run optimally. This research, therefore, aims to (i) describe the symptoms of blast disease in upland rice in Lombok, West Nusa Tenggara, (ii) determine the incidence of blast disease in upland rice in Lombok, West Nusa Tenggara, (iii) identify the pathogenic species cause blast disease in upland rice, in Lombok, West Nusa Tenggara.

MATERIALS AND METHODS

Blast disease survey
Samples of rice crops with blast symptoms were surveyed in Lombok, West Nusa Tenggara dryland cultivating rice crops. Samples were taken randomly from the dryland area cultivating rice crops, which found 50 locations potentially attacked by blast disease. At one location, three samples of rice crops with blast symptoms were taken randomized by walking zigzag, resulting in 150 samples of rice crops. Samples were labeled and stored in plastic bag sampling, isolated, identified, and sent to the laboratory.

Isolation and identification of pathogen causing blast disease in rice crops
Rice plant samples with blast symptoms were isolated and identified macroscopically and microscopically at the Integrated Laboratory of Mataram State Islamic University. Before isolation, the samples were cleaned under running water. Next, rice tissues were cut to a size of ±1 x 1 cm and were then sterilized with 70% alcohol for 1 minute and bleached (containing 5.3% sodium hypochlorite) for 2 minutes. Rice tissues were rinsed with sterile water three times and dried with thick, sterile tissues.

The fungus was isolated using the direct platting technique by lowering 4-6 pieces of dried plant parts on the surface of 2% media to which chloramphenicol (200 mg/L of medium) had been added. Next, 2-day-old isolates that showed hyphal growth were reisolated in sterile PDA media. Then, single spores isolation was conducted after 4-5 days.

One petri dish of pathogen isolates was multiplied into four isolates, so the total isolates were 600 isolates obtained from 150 samples multiplied by four replications. All inoculated media were then incubated at room temperature of 27-28°C. Next, each representative colony was separated into separate isolates (Gupta et al. 2020). Finally, the shape of the isolated colony was macroscopically observed, while the shape of the hyphae and spores was observed microscopically.

Molecular identification
The initial stage of molecular identification starts with DNA extraction. Hyphae from the edge of the colony taken from rejuvenated fungal cultures on PDA media aged three days were then put into a centrifuge bottle to be suspended with 100 μL PrepMan Ultra Reagent (PrepMan Ultra Protocol, Applied Biosystem, USA). The sample was vortexed for 30 minutes, then placed on a hot block at 95-100°C for 10 minutes and at room temperature for two minutes. In the next step, the sample was centrifuged at 10,000 rpm for two minutes, and then the pellet was taken as a DNA extract, which was used for further analysis (Raja et al. 2017).

The DNA extract obtained was used as a template for amplification using the PCR (Polymerase Chain Reaction) method using primers ITS 1 (forward primer) and ITS 4 (reverse primer). Next, DNA amplification of the 18S rRNA gene was carried out by PCR using primers Internal Transcript Spacer (ITS) 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3'), with a PCR product of 600 bp. The reaction was done using a Takara PCR Thermal Cycler Personal apparatus (Takara Bio, Otsu, Japan) with EX Tag (Takara Bio, Otsu, Japan) under pre-denaturation conditions of 94°C (4 minutes) followed by 35 cycles of 94°C denaturation (35 seconds), annealing 52°C (55 seconds), elongation 72°C (2 minutes) and post elongation 72°C for 10 minutes (Raja et al. 2017). Before sequencing, the PCR results were first electrophoresed using 1% agarose gel.

DNA sequence determination was carried out with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA, USA) according to the instructions on the tool and the PE Applied Biosystems Automated DNA Sequencer (model 3130xl, Applied Biosystems) (Kaynak-Onurdag 2016). The double helix DNA sequence was assembled and analyzed using Genetyx (version 11.0) and Genetyx-ATSQ (version 4.0) software (Genetyx, Tokyo, Japan) respectively, then compared with the same DNA sequence, which was taken from DDBJ/EMBL/GenBank via the program NCBI BLAST (Nishizawa et al. 2015).

Phylogeny analysis
The phylogeny relationship was then analyzed using the Mega 6.0 program and designed using the Maximum Parsimony (MP) method with a 1000x bootstrap (Khalimi

et al. 2019). Sequence data was saved in notepad in FASTA format and then analyzed using the Blast-WU facility, which is available online on the website www.ebi.ac.uk/Crustalw, by looking for similarities between sequences. The data from processing using CrustalW was then used to create a phylogeny tree using the MEGA data facility (Raja et al. 2017).

RESULTS AND DISCUSSION

Blast disease incidence in upland rice

Rice crops identified from 50 sampling points came from dryland spread across the Lombok region, West Nusa Tenggara, the agricultural land where most of the irrigation is rainfed. Symptoms of blast disease in rice crops were found at all sampling locations (Table 1). Several varieties of rice crops with blast symptoms are found at the location, including Inpari 32, Inpari 34, Inpago 10, Inpago 9, Inpago 8, and Upland rice. All of these varieties are classified as susceptible to blast disease.

The range of blast disease attacks in rice crops is from 2 to 76%. The highest attack rate was found in Kuta, with a percentage of $76.67 \pm 40.41\%$. In comparison, the six locations with blast symptom attack rates above 30% were found in Rembitan 1, Mertak, Gumantar, Akar-akar, Anyar, and Bayan. Apart from these six locations, most blast attacks are below 30%. Therefore, all dryland samples in Lombok, West Nusa Tenggara, found rice with blast symptoms.

Symptoms, macroscopic and microscopic identification of blast disease in upland rice

Blast disease symptoms that attack rice crops in dryland Lombok are found on the leaves, panicles, and grains of rice crops, as shown in Figure 1. The macroscopic characteristics of upland rice leaves affected by blast disease are observed from brown spots and points like a rhombus; the edges are brown and grey or whitish in the center of the spots.

Macroscopic morphology of blast disease in rice crops planted in the media formed a white mycelium, the front and back surfaces of which are also white and white-grey; the front surface is white while the back is brown and is colored white-brown, the front surface is white but predominantly dark brown, the back surface is dark brown (Figures 2). Lateral mycelial growth with a fine structure has macroconidia, microconidia, and chlamydospores. Based on microscopic observation under a microscope, it was found that the hyphae's shape was septate, conidiophore structure with autophagic cell death, long pyriform microconidia, and long pyriform macroconidia.

Molecular identification of blast disease in upland rice

Moreover, the molecular identification shows that Lombok Blast isolate has high similarity, observed from the comparison with the database from GenBank, and among the isolates that had high similarity consisted of *Pyricularia* sp. Strain F2, *Pyricularia oryzae* Isolate Ic1, *Pyricularia oryzae* Isolate CBS 433-70, *Pyricularia oryzae* Isolate lb4, and *Pyricularia oryzae* Isolate RB30 with

similarity percentages of 100%, 99.62%, 99.62%, 95%, and 99.06% respectively (Table 2).

Analysis of the closeness of the tree phylogenetic relationship is shown in Figure 3. The blast disease analysis on the Lombok isolate showed a similarity percentage of 99.62% with *Pyricularia oryzae* Isolate Ic1 (OQ552821.1). Therefore, based on this similarity percentage, it can be concluded that the blast disease Lombok isolate species is *Pyricularia oryzae*.

Table 1. Incidence of blast disease in upland rice in several dryland locations, Lombok, West Nusa Tenggara, Indonesia

Sampling location	Disease incidence (%)	Varieties of rice
Montong Sapah	2.33	Inpari 32
Kabul	4.00	Inpari 32
Pelambik	5.67	Inpari 32
Ranggagata	3,33	Inpari 32
Darek	5.67	Inpari 32
Batu Jangkih	4.00	Inpari 34
Montong Ajan	2.33	Inpari 34
Pandan Indah	2.00	Inpari 34
Srage	5.67	Inpari 34
Selong Belanak	9.00	Inpago 9
Mekar sari	2.67	Inpago 9
Banyu urip	5.67	Inpago 9
Kateng	4.67	Inpari 32
Mangkung	10.67	Inpari 34
Bonder	8.33	Inpago 10
Setanggor	2.33	Inpago 10
Penujak	2.67	Inpago 10
Batu Jai	8.00	Inpari 32
Tanak Rarang	6.67	Inpari 32
Tumpak	3.67	Inpago 8
Prabu	11.67	Inpago 8
Kuta	76.67	Upland rice
Rembitan 1	55.00	Inpari 32
Rembitan 2	28.33	Inpari 32
Sukadana	10.00	Upland rice
Mertak	56.67	Upland rice
Pengengat	12.33	Inpari 32
Teruwai 1	14.33	Inpari 32
Teruwai 2	20.67	Inpari 32
Gapura	19.33	Inpari 32
Kawo 1	23.33	Inpari 32
Kawo 2	11.00	Inpari 32
Segala anyar	6.67	Inpago 8
Sengkol 1	23.33	Inpago 8
Sengkol 2	22.67	Inpari 32
Pengembur 1	18.33	Inpari 32
Pengembur 2	11.67	Inpari 32
Pengembur 3	10.67	Inpago 8
Ketara	16.33	Inpago 8
Tanak Awu	20.00	Inpago 8
Gumantar	31.67	Inpari 32
Pendua	15.33	Inpari 32
Salut	27.67	Inpari 32
Akar-akar	38.33	Upland rice
Anyar	38.33	Upland rice
Bayan	43.33	Upland rice
Senaru	2.33	Inpari 32
Karang Bajo	10.00	Inpari 32
Loloan	18.33	Upland rice
Sambi Elen	11.67	Upland rice

Figure 1. Symptoms of blast disease in different part tissue of upland rice, Lombok West Nusa Tenggara: A. Straw, B. Leaf, C. Panicle, D. Grain
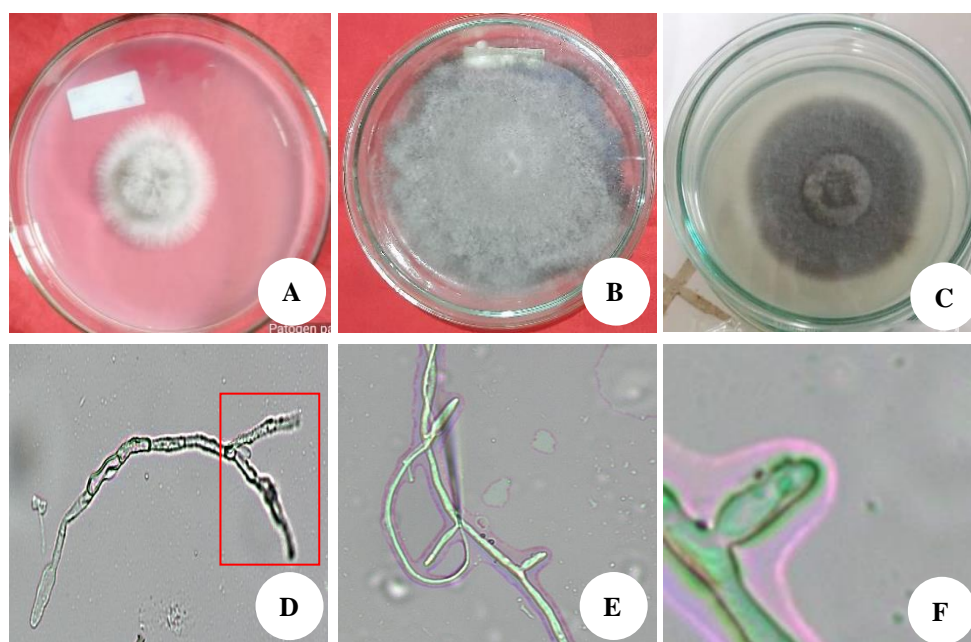

Figure 2. Morphology of isolate Blast Lombok *Pyricularia oryzae* causing blast disease in upland rice, Lombok, West Nusa Tenggara. A-C. Colony, D. Structure of conidiophore with autophagic cell death, E. Conidiophores and conidia, F. Conidia

Table 2. Percentage of similarity of isolate Blast Lombok causing blast disease in upland rice, Lombok, West Nusa Tenggara, with several homologous sequences in the GenBank

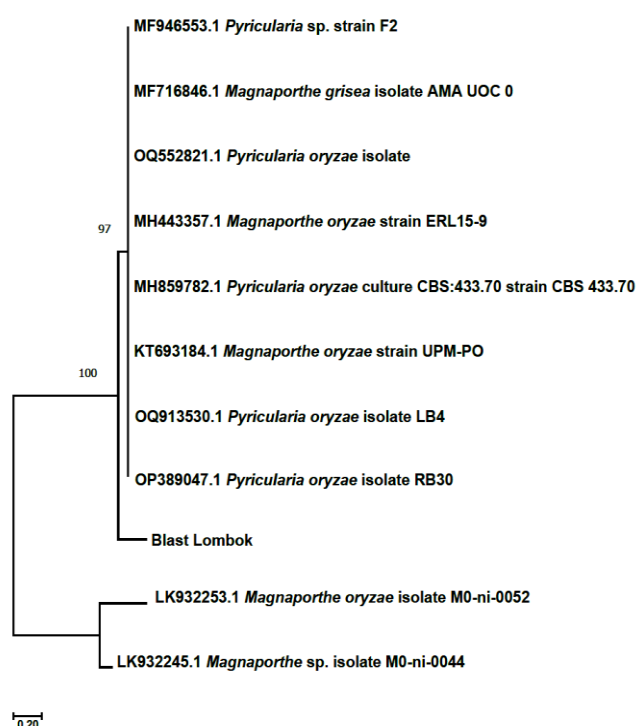| Isolates	Similarity (%)
Pyricularia sp. Strain F2	100.00
Magnaporthe grisea Isolate AMA UOC 0	96.00
Pyricularia oryzae Isolate Ic1	99.62
Magnaporthe oryzae Strain ERL15-9	99.61
Pyricularia oryzae Strain CBS 433.70	99.62
Magnaporthe oryzae Strain UPM-PO	99.81
Pyricularia oryzae Isolate LB4	95.00
Pyricularia oryzae Isolate RB30	99.06
Magnaporthe oryzae Isolate M0-ni-0052	98.70
Magnaporthe sp. Isolate M0-ni-0044	99.62

Figure 3. Phylogenetic relationship tree of isolate Blast Lombok in upland rice, Lombok, West Nusa Tenggara, with several isolates of fungi based on 18S rRNA gene constructed by using neighbor-joining method and bootstrap (1000 replicates) analyzed by using Kimura 2-parameter model (MEGA 11 software)

Discussion

Dryland is known for its limited water supply. As a potential area for dryland development, West Nusa Tenggara has developed many types of cultivated plants; rice is only one food plant that local farmers have long cultivated. In dryland, local farmers generally cultivate rice varieties resistant to extreme conditions. However, the probability of disease attacks such as blast disease in rice crops is high because of environmental conditions in dryland areas with extreme temperatures and relative humidity. These results also align with research by Paradisa et al. (2018) study location in dryland.

Observation from 50 Lombok dryland locations showed that all the rice crops at those sites had blast symptoms. Dryland areas can increase the risk of spreading disease in plants; temperature and climate are the two leading factors in increasing the spread of blast disease in rice crops (Kirtphaiboon et al. 2021). Blast disease is found in wetlands with water and nutrients available in ideal quantities, and blast disease in rice crops is found in drylands. A dryland with limited water availability is more at risker of being attacked by blast disease, which can reach a 60% yield loss risk (Amir et al. 2023).

Seven locations with the highest blast attacks were found in Kuta, Rembitan, and Mertak, the dryland areas of Southern Lombok. At the same time, Gumantar, Anyar, Bayan, and Akar-Akar are the dryland areas in the North (Table 1). These attacks are because the area is rainfed dryland or has low rainfall intensity and temperatures classified as suboptimal for blast disease sporulation. Rainfall in 2020 reported by the Central Statistics Agency (Statistic Indonesia 2021) for North Lombok Regency as a whole was 11 mm, as well as Central Lombok Regency in the Pujut area showed an average rainfall of 12 mm in 2016 with temperature intervals in the region between 27-32°C (Statistic Indonesia 2016). Those conditions are potentially conducive to blast disease development.

Moreover, several studies reported the characteristics of land, which generally have relatively high air humidity, such as 90-92% (Zewdu 2021) and limited water availability, have a high risk of blast disease. Aung et al. (2018) revealed that water availability in and likely associated changes to the apoplastic environment determine the success of pathogenic microbes' attack potentials. The following environmental conditions also indicate limited food sources for living organisms around the plant, so the probability of attack and spread of blast disease in rice crops can also increase significantly, especially if the temperature range is 27-32°C, which is the optimal for the diseases but suboptimal for growth and development during blast disease life cycle (Rajput et al. 2017).

Furthermore, blast disease attacks in rice crops at all dryland locations show the same symptoms. Morphologically, the most typical characteristics of blast disease shown in dryland rice crops are brown spots shaped like rhombuses (Figure 1). Kuswinanti et al. (2023) also obtained the same symptom characteristics in *Pyryicularia orizae*. Other studies also report on the blast disease *Pyricularia oryzae*, which can attack all rice plant tissues, including leaves, stems, panicles, and grains (Asibi et al. 2019), as shown in Figure 1. Furthermore, blast disease cultured in agar media formed fine mycelium, which is white-grey, and colonies from the center to the sides of the petri dish, which can be seen from above and below the surface of the petri dish (Figures 2A-2C). These results align with research by Longya et al. (2020), who examined the morphological characteristics of *Pyricularia oryzae* with different mycelial colors, such as cream, fluffy, densely, grey, and flat.

Therefore, referring to the above explanation, it concluded that the cause of blast disease symptoms in rice crops in dryland, Lombok, West Nusa Tenggara (NTB) is *Pyricularia*

oryzae (Table 2, Figure 3). This report is the first report in West Nusa Tenggara that one of the causes of damage to rice plant tissue is the blast disease *Pyricularia oryzae*. Several previous research in Indonesia reported that the cause of blast disease in rice crops in Bali by *Magnaporte oryzae* (Sugiarta et al. 2021), *Pyricularia* oryzae found in West Java, Lampung, South Sumatra, West Kalimantan, and Bali (Kadeawi et al. 2021), and *Pyricularia grisea* found in East Kalimantan (Sopialena et al. 2019).

The most common life cycle found in the pathogen *Pyricularia oryzae* is asexually by forming spores. These spores are called conidia and are produced abundantly on conidiophores (Asibi 2019). Boddy (2015) and Zhang et al. (2017) reported that *Pyricularia oryzae* form conidia and conidiophores to survive and spread over long periods. The pathogen infects plant tissue by forming three-septated conidia, which are released by conidiophores; the conidia move to the surface of uninfected leaves through water splashes or wind blows.

Based on molecular identification, the DNA chromosome length of blast disease Lombok was ±550 bp (Figure 4). Furthermore, the phylogeny tree analysis (Figure 3) shows the blast disease in rice crops in dryland has a close relationship with *Pyricularia oryzae* strain F2 (MF946553.1) with a percentage similarity of almost 100%. In Thailand, Longya et al. (2020) obtained identical results for all isolates tested, namely with a chromosome length of 497 bp in *Pyricularia oryzae* when DNA sequencing using ISSR and SRAPS markers. Likewise, according to the report by Zheng et al. (2018), when rearranging several *Pyricularia oryzae* genotypes, one of the chromosome lengths obtained from the analysis was 567 bp and those chromosome wavelengths were similar in this study.
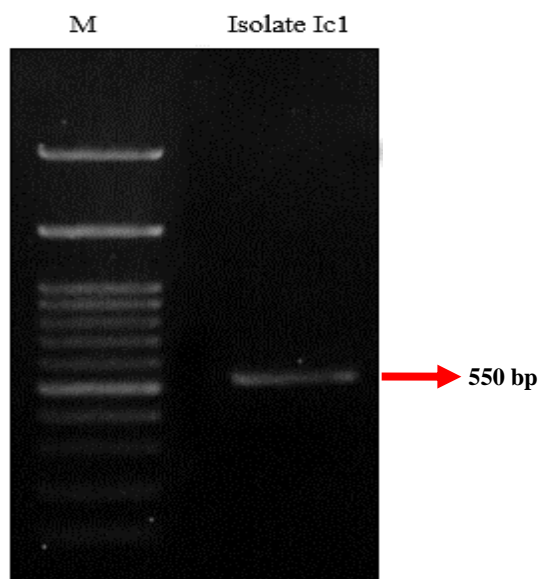
Therefore, this study concludes that (i) typical symptoms of rice crops in dryland, Lombok, NTB, which are attacked by blast disease are brown leaf spots shaped like rhombuses (ii) the incidence of blast disease in rice crops in dryland, Lombok, NTB is found in all location; with a minimum incidence range of 2% and a maximum of 70% (iii) the pathogen that attacks rice crops in dryland, Lombok, NTB is *Pyricularia oryzae* In the PDA experiments, Lombok Blast isolate has white colonies, mycelium growth is lateral, and the mycelium structure is smooth. The fungi produce fine mycelium consisting of microconidia, macroconidia, and conidiophores. Based on 18S rRNA gene analysis, the Lombok Blast isolate had a 550 bp DNA fragment, which was 100% identical to *Pyricularia oryzae* Strain F2 (MF946553.1).

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