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BAB V KESIMPULAN UMUM

Berdasarkan hasil penelitian yang telah dilakukan maka dapat disimpulkan bahwa:

1. Bakteri penyebab penyakit busuk batang jagung yang ditemukan menginfeksi di Sulawesi Selatan, Sulawesi Barat, Gorontalo, Kalimantan Timur dan Yogyakarta teridentifikasi *Dickeya zeae* dan *Dickeya* sp dengan karakter morfologi berupa koloni bulat, elevasi cembung, berwarna putih abu-abu, sel bakteri berbentuk basil tunggal. Karakter fisiologis berupa gram dan oksidase negative, katalase dan indol positif, oksidatif fermentatif, pektolitik, mampu tumbuh hingga suhu 39^0C dan NaCl 5%, produksi beberapa enzim diantaranya lesitinase, protease, fosfatase, sensitif terhadap antibiotik eritromisin dan mampu memanfaatkan gula seperti D-arabinosa, Laktosa, D-Melibiosa, D-Raffinosa, Mannitol, Mso-Tartrate dan Myo-Inositol tetapi tidak menggunakan D-Tartrate, inulin, dan 5-ketoglukanat sebagai satu-satunya sumber karbon.
2. Terdapat satu dari sembilan isolat bakteri yang mempunyai virulensi tertinggi dan masa inkubasi tercepat (*D. zeae* strain #064) dan dijadikan sebagai isolat standar untuk pengujian lebih lanjut
3. Karakter patologi *D. zeae* yakni perkembangannya cepat, kisaran inang luas, tular tanah, bertahan hidup pada tanah, kadar lignin batang berpengaruh terhadap insidensi penyakit serta inokulasi patogen yang tepat untuk bakteri ini ialah injeksi ke batang.
4. Galur MTD1-1 dan MTD1-7 konsisten memiliki nilai LDBKPP rendah dan indeks proteksi lebih dari 50% pada dua musim tanam dan galur-galur tesebut telah diselfing hingga terbentuk galur S3. Galur yang terbentuk berpotensi digunakan sebagai bahan perakitan varietas unggul baru jagung yang tahan busuk batang.

LAMPIRAN

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The presence of bacterial stalk rot disease on corn in Indonesia: A review

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Abstract. Bacterial stalk rot disease in corn results in a significant reduction in yield due to the interruption of the flow of nutrients from the roots to other parts of the plant. Pathogenic bacteria infect the inner tissue of the stalk until it rots. This disease has been reported to attack corn crops in Asia and Europe such as India, Korea, Thailand, Philippines, Nepal, Mexico, Serbia, and China. In Indonesia, this disease was first reported to attack corn in the West Sulawesi region by the Mamuju Class II Quarantine Station. The results of molecular identification indicated that this disease is caused by the bacterium *Dickeya zeae*, previously known as *Erwinia chrysanthemi* pv. *zeae* that previously reported attacked pineapple and aloe vera in Indonesia. The potential for economic losses due to this disease is quite high, so appropriate and efficient control measures are needed. Based on those, this research study about the symptom, the characteristic of the bacteria agent caused the stalk rot disease, the distribution and the impact to the maize production in Indonesia.

1. Introduction

Corn (*Zea mays* L.) is a cereal plant that is widely cultivated by farmers in Indonesia to fulfill food and animal feed needs. Corn is currently a strategic national commodity and in the economic nomenclature of food crops in Indonesia, corn is the second important commodity after rice [1]. National corn production in 2019 reached 22.59 million tons with the main producing areas or centers of corn harvesting area in Indonesia distributed in ten provinces with a total contribution of 83.53% to Indonesia's total harvested area [2]. The ten regions are East Java, Central Java, Lampung, North Sumatra, South Sulawesi, West Nusa Tenggara, West Java, North Sulawesi, Gorontalo and South Sumatra.

Corn harvested area for the 2015-2019 period continues to increase by an average of 4.38% per year [2]. This is of course supported by several factors including natural resources, agroecological environment, and government policies to support increased corn production. Currently, the increase in corn production continues to be driven through a Corporate Food Crop Area Development Program (ProPaktani) to increase production and exports so that the agricultural sector becomes stronger as a support for the national economy. Another program is the procurement of seeds through the Seed Independent Area Model under the coordination of IAARD and Seed Independent Village under the coordination of the Directorate General of Food Crops [1]. Hipi et al (2015) in [3] stated that fostering farmer groups in the Seed Independent Village program in several provinces succeeded in producing

quality hybrid corn seeds. This certainly has an impact on surrounding farmers because it can reduce the cost of corn production in the form of lower seed prices because it is produced from the local area.

Efforts to increase corn production continue to be carried out to meet the growing demand for corn as a raw material for livestock. Corn is mostly chosen as a constituent of livestock feed because it is easy to digest, palatable and does not contain anti-nutrients. Corn also contains xanthophylls which [4]. The sustainable corn self-sufficiency program launched by the government is an ideal condition because Indonesia has natural resources and a supportive agroecological environment [5]. However, efforts to increase production are inseparable from biotic and abiotic environmental constraints that reduce productivity, including climate disturbances, use of poorquality seeds and attacks by plant pest organisms.

In Indonesia, it is reported that several main diseases that infect corn plants include downy mildew, maydis leaf blight, and leaf rust. Evaluation of the resistance of new high-yielding varieties to these three diseases is a major prerequisite for the release of new varieties [6]. However, at this time, pathogenic infections are starting to be found that cause tissue rot in the stalk with soft rot and slimy characteristics, plants wilt and heavy attacks cause plants to die. The literature study conducted showed that the symptoms of the disease were stalk rot caused by infection with the *Dickeya zea* bacterium, previously known as *Erwinia chrysanthemi* pv. *zeae* which is one of the most important diseases of corn in the world.

Based on those, the ethological study about this disease in Indonesia need to be concern. The information about the symptom, pathogen characterization, the distribution and the impact to the maize production in Indonesia must be done. The characterization of bacteria that cause stalk rot disease in corn from pathological, biochemical and molecular characteristics to be used as a basis for handling corn stalk rot disease. Pathological characteristics were carried out to see the virulence of bacterial isolates found from corn cultivation in infecting and causing disease as well as the resistance of several existing varieties of corn to stalk rot disease. Biochemical characteristics were carried out to see the physiological properties of the bacterial isolates that cause stalk rot disease to determine the characteristics and specifics by looking at the enzyme activity. Molecular characteristics to obtain more accurate information on the bacterial species found.

2. Symptom of Disease

Symptoms of corn stalk rot disease caused by bacterial infection are generally found to be maceration of the stalk and a change in the color of the infected tissue to brown and softened rot, emitting an unpleasant odor and eventually the plant collapses [7-9]. The presence of an unpleasant odor is one of the things that distinguishes the symptoms of stalk rot disease caused by bacteria and fungi [10].

Corn stalk rot disease is economically detrimental because of the interruption of the flow of nutrients to plant tissues so that the filling of the cobs is not perfect and even severe infections can kill plants before physiological maturity [10]. Furthermore, [9] and [11] found that severe infection conditions caused infected plants to collapse, resulting in a significant reduction in yield (Figure 1). Severe infections are usually found when climatic conditions with high temperature and humidity, such as in tropical and sub-tropical regions, occur sporadically [12]. Symptoms of the disease found in one of the corn development areas in South Sulawesi are the same as the description above, if the infected corn stalk is split, the color changes to brown and emits an unpleasant odor, the plant dies as a whole (Figure 2).

Figure 1. Symptoms of bacterial stalk rot disease on corn found in Korea (Myung et al., 2010)

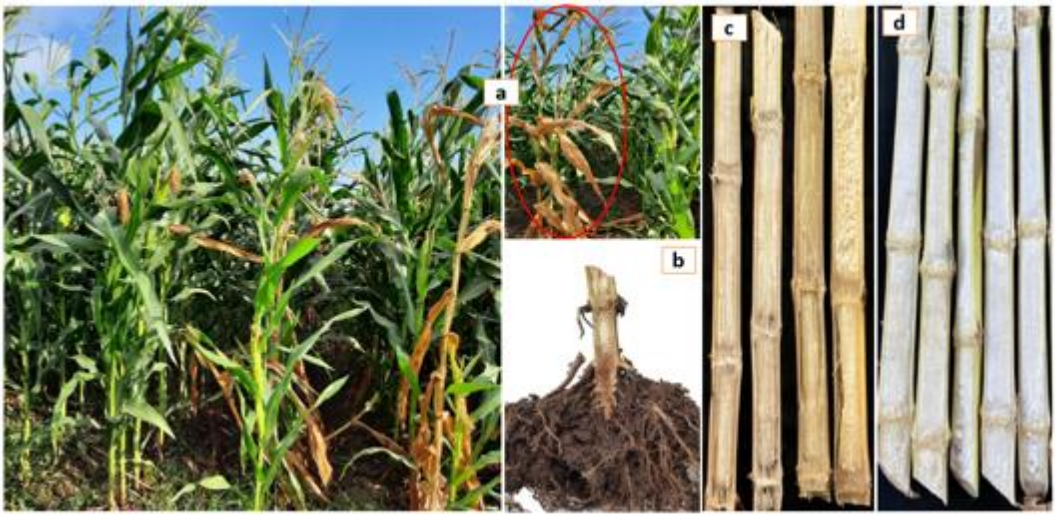

Figure 2. (a) Symptoms of plants infected with bacterial stalk rot disease found in Kab. Gowa, South Sulawesi in 2021; (b) Maceration of infected stalks from below the soil surface; (c) discoloration of infected stalk tissue; (d) healthy stalk tissue (Private Collection).

3. Characteristics of the Pathogen

Identification of bacteria in general can be done in several ways, including by observing morphology, biochemical properties, or by using molecular equipment. Observation of bacterial morphology can be done macroscopically and microscopically by observing the shape of the colony, such as point-shaped, round, irregular like roots and filamentous or threaded and coiled. Colony edges can be whole, wavy, split, serrated, threaded and curly. Colony color consisted of whitish, yellowish, reddish, brown, orange, pink, green and purple. Colony elevations include flat, horizontal, curved and convex. The colony structure is smooth, shiny, rough, wrinkled or curly [13].

Dickeya zeae which is one of the bacteria that causes corn stalk rot disease that has been widely reported so far. Specific identification of the presence of this bacterium is its ability to produce indigoidine, a blue pigment that is insoluble in water. *Dickeya zeae* is the only species in the bacterial genus Erwinia that is able to give a blue color to bacterial colonies as a chemotaxonomic property for rapid identification [14].

Morphological identification carried out by [11] against *D. zeae* found the characteristics of rod-shaped and gram-negative bacteria. Size varies from 0.8-3.2 x 0.5-0.8 m (mean 1.8 x 0.6 m) and contains 3-14 peritrichous flagella. These bacteria were producing white, slimy and shiny colonies on King's B media, while on Nutrient Agar media the bacterial colonies were gray and slightly prominent [9] (Fig. 1A and 1B).

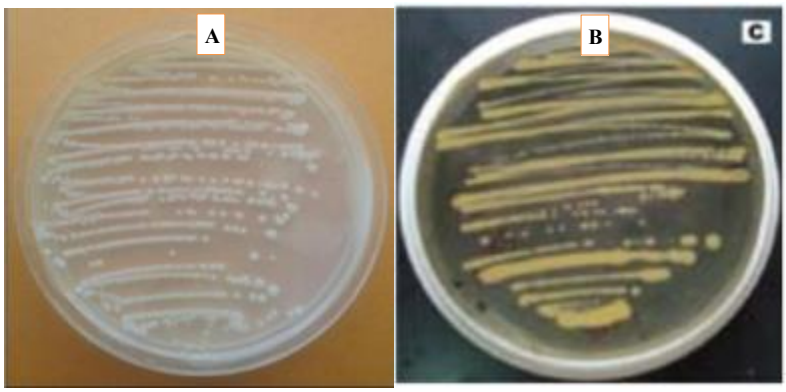

Figure 3. (A) Single colony culture of *D. zeae* isolated from corn stalks and purified on King B media [11] and *D. zeae* culture on Nutrient Agar media [15].

The morphological characteristics of bacteria are considered less effective because of their large potential for contamination [16]. Another drawback of this method lies in the nature of the bacteria obtained. This is because many bacteria have the same colony shape and color. Therefore, identification of bacteria needs to be done in several ways including biochemical, pathological and molecular characteristics. In particular, bacteria that cause stalk rot in corn have been identified by several researchers, both from biochemical characteristics and molecular detection. However, information regarding this bacterium in corn plants has not been widely reported in Indonesia.

3.1. Characteristics of Pathology

Bacterial pathogenicity is the ability of a pathogenic bacterium to cause disease. [17] stated that the pathogenicity of each pathogenic agent is also closely related to its ability to produce enzymes, toxins and the ability to overcome the host's immune systole. In addition, bacterial pathogenicity is a multifactorial process, successful infection requires temporal coordination of survival and expression of virulence genes [18].

Dickeya zeae is also known as Erwinia soft rot bacteria which belongs to the Enterobacteriaceae family. The bacteria are gram negative rod-shaped with peritrichous flagella [19]. *D. zeae* produces several virulence factors including phytotoxic zeamines and extracellular enzymes including pectinase, protease, feruloyl esterase and cellulase enzymes which collectively contribute to bacterial infection [20-22]. These bacteria secrete enzymes that destroy plant cell walls, causing cell lysis and the release of cellular fluid in the form of characteristic rot symptoms [23].

Characterization of the bacterial pathology of corn stalk rot disease is usually carried out by testing the decay activity of potato tubers, hypersensitivity reactions on tobacco leaves, pathogenicity tests on corn plants and appropriate inoculation methods. Bacterial isolates that have high virulence can be used to screen corn resistance to stalk rot disease. The high virulence of the bacteria is indicated by its ability to cause soft rot symptoms that are getting faster in all infected plant tissues [24]. Soft rot bacteria from the *Dickeya* sp. will enter the potato tuber through lenticels, stolon and or wounds and the infection can spread to all parts of the plant [19].

Testing the pathogenicity of plant pathogens must be supported by appropriate inoculation techniques. [25] tested 4 methods of inoculation of *D. zeae* on corn in India and the results showed that the method of stalk injection and immersion of plant roots in bacterial suspension caused the greatest incidence of disease. However, the root immersion method with bacterial suspension was considered less effective in testing plant resistance to stalk rot disease in plantations and the chance of plant damage due to carelessness during root removal was quite high. [26] reported the results of testing 6 methods of inoculation of *Dickeya dadantii* bacteria in causing sorghum stalk rot disease showing that the use of the tootpick inoculation method resulted in a fairly high incidence of disease.

3.2. Biochemical Characteritics

Biochemical tests help identify different bacterial species based on different biochemical activities. Differences in carbohydrates, proteins, fat metabolism, production of certain enzymes, ability to utilize certain compounds etc., help to identify microorganisms. Several researchers have identified biochemically *D. zeae* isolated from corn plants and biochemical characteristics such as facultative and non-fluorescent anaerobic, pectinolytic in potato tubers, causing hypersensitivity reactions in tobacco leaves, producing catalase and lecithinase, not producing oxidase or arginine dehydrolase, capable of reducing nitrate and can grow at a temperature of 37^0C. Among the 23 strains studied, 11 grew well under 5% NaCl, while the growth of the other 12 strains was inhibited, indicating that some bacterial strains were more tolerant of salt [27]. The strain formed intensive "fried egg" red colonies with a diameter of 1.5 mm on potato dextrose agar (PDA) medium (Table 1). These growth characteristics are described as typical for bacteria belonging to the genus Dickeya.

Table 1. Physiological and Biochemical Characteristics of *Dickeya zeae* isolated from maize and the reference strain.

Characteristic	Strains of Dickeya Zeae (n=23)	Reference strains		
		Dickeya spp. (KBI 05)	Pcc (KFB 85)	Pba (KFB 07)
Characteristic				
Gram reaction	-	-	-	-
HR on tobacco leaves	+	+	+	+
Oxidase activity	-	-	-	-
Catalase activity	+	+	+	+
Lecithinase activity	+	+	-	-
Fluorescence on KB	-	-	-	-
Glucose metabolism	OF	OF	OF	OF
Potato soft rot	+	+	+	+
Growth at 37 ^{o}C	+	+	+	-
Growth in 5% NaCl	v	+	+	+
Growth on Logan's medium	$+^a$	$+^a$	$+^b$	$+^c$
"Fried egg like colories on PDA medium				
Nitrate reduction	+	+	+	+
Pathogenicity assay	+	+	-	-

Legend : + indicates positive reaction; indicates negative reaction; v, indicates variable reaction; OF, oxidative-female metabolism of glucose; $+^a$, intensive red colonies 2 mm in diameter, $+^b$, smaller (1,5 mm) colonies with pink center, $+^c$, small (<1.5 mm) white-grey colonies
Source : [27]

Other researchers reported the same characteristics of the bacteria causing corn stalk rot found in Korea. These bacteria are gram-negative, oxidase negative, catalase positive, fermentative, rod-shaped, motile, and facultative anaerobes. The results of biochemical tests performed using the Biologic Microbial Identification Systalk, version 4.2 (Biolog Inc., Hayward, CA) showed that all isolates had a similarity index of 0.65 to 0.73 with *D. zeae* [9].

Dickeya zeae in addition to infect corn was also found to infect horticultural crops such as pineapple. The results of the identification of bacteria that cause rot in pineapples identified as *D. zeae* were reported to be gram negative, soft rot, facultative anaerobes and virulent [28].

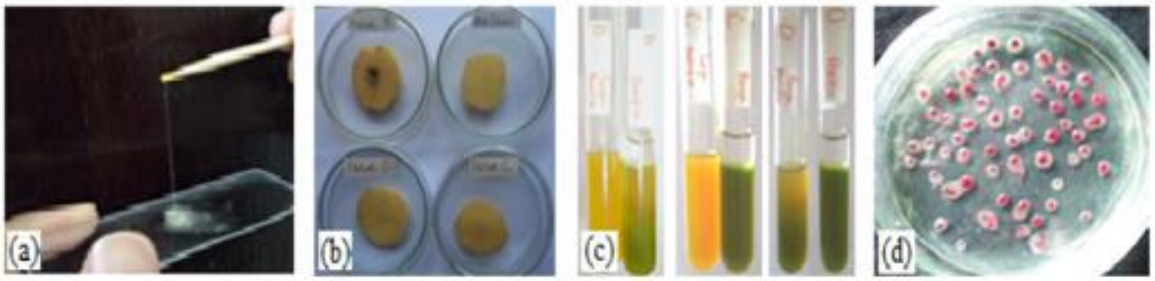

Figure 4. (a) Gram test results using 3% KOH, (b) and (c) Potato tuber rot test results (left) and O/F test on bacterial isolates B, C and D (right) Test results using TZC media on isolate B, (d) The results of the test using TZC media on isolate B [28].

3.3. Moleculer Characteristics

Molecular characterization is an important tool for the identification of plant pathogens with the help of locus/gene-specific primers [29-30] . Strategies that are widely used for rapid detection of pathogens include screening target DNA sequences or using probes to develop DNA markers of infecting pathogens [31], as well as molecular detection with simple Polymerase Chain Reaction (PCR) and its development.

Molecular test with PCR is a technique of DNA synthesis and amplification in vitro. This technique was discovered by Kary B. Mullis and F. Faloona in 1985. PCR is based on the enzymatic amplification of DNA fragments using two complementary oligonucleotide primers with the 5' ends of both strands of the target sequence. These oligonucleotides are used as primers (PCR primers) to allow DNA templates to be copied by DNA polymerase [31].

The genus Dickeya as a bacterium that causes soft rot disease in many plants consists of six species, namely *D. dianthicola, D. dadantii, D. zeae, D. chrysanthemi, D. dieffenbachia* and *D. paradisiaca* [23]. The results of biochemical characteristics conducted by [32] stated that *D. dadantii* and *D. zeae* are in the same phenon so that molecular identification is needed for more accurate determination of pathogenic species. Their research to identify the bacteria that causes soft rot in pineapple plants used three different genes, namely 16S rDNA, recA, and dnaX. The 16S rDNA approach is considered as one of the most widely used standard techniques for inferring phylogenetic relationships among bacteria but sometimes it is not sufficient to distinguish closely related species. While recA and dnaX genes have been shown to be strong markers for inferring bacterial phylogeny and have been used successfully to differentiate Dickeya species; [23].; [33-34]; [13].

The use of specific primers for the detection of *D. zeae* was first carried out by [35] used a specific primer set (ADE-1, ADE-2) to detect 78 strains of *D. zeae* and all markers showed a specific band of 420 bps (Figure 3). Until now, this primer has been widely used for the detection of *D. zeae* in several commodities such as pineapple, aloe vera and orchids.

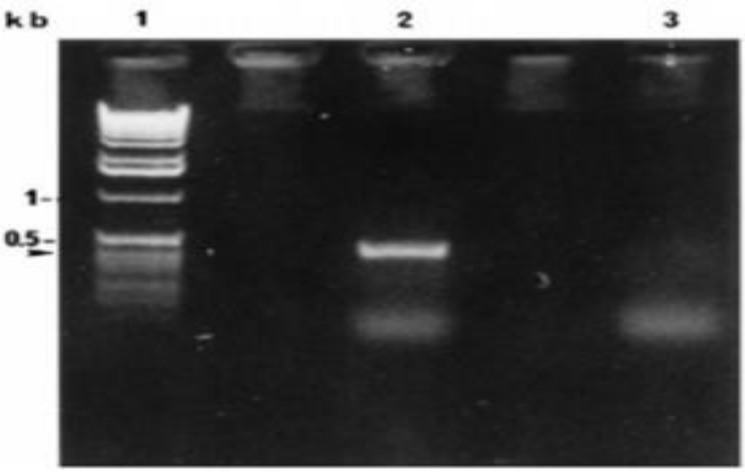

Figure 5. Results of PCR amplification using specific primers ADE1 and ADE2. The PCR products (after 25 cycles) were separated by electrophoresis on 1% agarose gel. Lane 1, 1-kb DNA ladder; lane 2, *E. chrysanthemi* 3937. The arrow direction indicates the position of the 420-bp amplified fragment [36].

Molecular identification of *D. zeae* carried out by [9] isolated from corn plants in Korea showed the results described in the following phylogenetic tree (Figure 4). There are two isolates isolated in Korea, namely *D. zea* BC2879 and *D. zeae* BC2880 which have a close relationship with the isolates *D. zeae* IPO649, *D. zeae* NCPPB1863 and *D. zeae* NCPPB2538. Meanwhile, the phenotypic test and amplification of the specific 420-bp fragment in the PCR test conducted by [27] showed that 7 isolates of corn stalk rot were included in the Dickeya genus based on phylogenetic analysis based on gene sequence results using recA. Using ERIC-PCR analysis seven different genetic profiles were obtained, indicating the presence of genetic diversity in the population of this pathogen in Serbia.

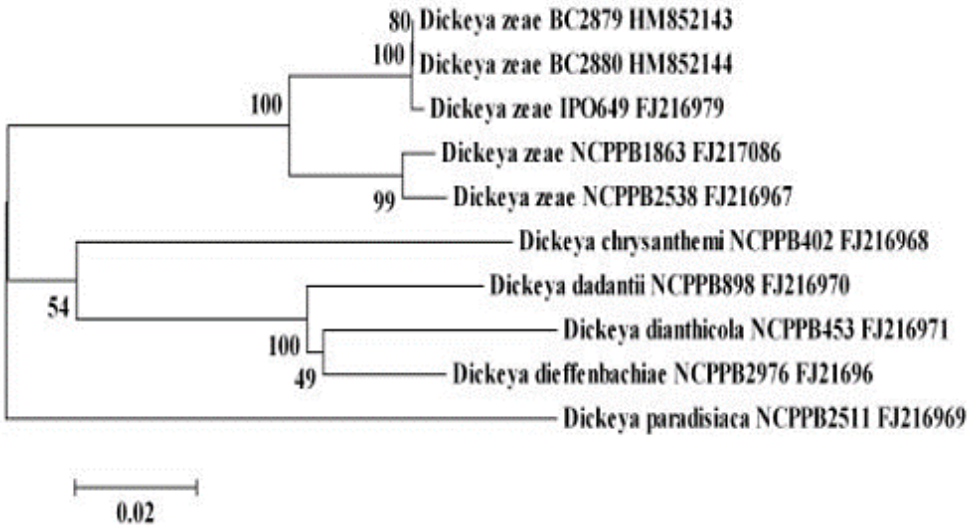

Figure 6. Phylogenic tree of PCR test results of 2 isolates of corn stalk rot pathogen found in Korea [9].

4. Disease Distribution

This maize stalk rot disease firstly reported by Prasad in 1930. The identification showed that this disease caused by *Erwinia dissolvens*. However, the characteristic tends to *Erwinia chrysanthemi*. The outbreak of this disease happened in Himachal Pradesh, 1969. The pathogen spread form one to other plant through the rain and the water flow.

This bacterium has long been found to infect corn plants in the Philippines with high attack intensity [10]. *D. zeae* was also reported to attack hybrid and composite corn in four corn growing areas in India with an average disease incidence of 96.65% [25]. Apart from these two countries, *D. zeae* was found to infect corn crops in Nepal, Serbia, China and Mexico in the last 10 years [27]; [36]; [12]; [8]. Initial infection in Shanghai China was found to attack sweet corn [8]. Until now, information regarding the detailed identification of bacteria that cause stalk rot disease in Indonesia is still very limited. Stalk rot disease that has been reported in Indonesia is caused by the fungus *Fusarium* spp. [37]. In the Minister of Agriculture No. 25 of 2020, this bacterium is classified in OPTK A2, which means that it is already present in Indonesia, but is limited to certain areas. The presence of *D. zeae* has been found to attack aloe, pineapple and corn plants in Indonesia [28]; [38-40]. This bacterial infection was found to attack pineapple plantations in the Lampung region and the host range test conducted by [32] showed that the isolates of *D. zeae* bacteria isolated from pineapples and inoculated on corn plants showed symptoms of stalk rot. The presence of *D. zeae* infecting corn plants in the West Sulawesi region was first reported by the Mamuju Class II Quarantine Station in 2019. The results of molecular identification showed that the bacteria found was *D. zeae* [40]. Meanwhile, other maize production areas have not do the identification and the survey about the disease distribution.

5. The Impact to the Maize Production in Indonesia

This maize stalk rot disease caused yield loss production directly by affect the plant physiological. This disease attack will caused the plant collapse and affect to the economic value [41]. High humidity factors and low oxygen levels cause high disease incidence and spread, pathogenic infections increase because they are facultative anaerobes (Perambelon, 2002 in [19]). This pathogen can be transmitted through the soil [42], but the inoculum can persist in infected plant residues in the soil for 270 days [43]. These bacteria can move in the soil up to a distance of 10 m through free water so that they can infect surrounding plants. In addition, the spread of bacteria can also be assisted by vectors in the form of insects from infected plants to healthy plants and can appear in aerosols formed by impaction of rain on symptomatic plants. These bacteria can also survive in surface water and can be spread through irrigation water (Lauria et al. 2008 in [19]). Another factor that triggers the development of this pathogen in agricultural land is numerous host plant.

6. Conclusion

The maize stalk rot caused by bacteria need to be concern because of the economic loss impact. The bacteria that caused stalk rot disease in corn reported from several countries are generally identified as *Dickeya zeae*, including those found in one of the corn development areas in Indonesia. The bacteria are gram negative, facultative anaerobes, produce catalase and lecithinase, do not produce oxidase or arginine dehydrolase, are capable of nitrate reduction and can grow at 37°C, producing several plant cell-degrading enzymes, pectinolytic properties on potato tubers and causing hypersensitivity reactions in tobacco leaves. The spread of bacteria through the soil, the ability to survive on infected plant residues for a long time and a wide host range can be a threat factor in the spread of this pathogen in Indonesia.

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Morpho-physiological and molecular characteristics of bacteria causing stalk rot disease on corn in Gorontalo, Indonesia

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Abstract. *Suriani, Patandjengi B, Muis A, Junaid M, Mirsam H, Azrai M. 2023. Morpho-physiological and molecular characteristics of bacteria causing stalk rot disease on corn in Gorontalo, Indonesia. Biodiversitas 24: 1749-1758.* Stalk rot disease was observed in corn in Gorontalo with typical symptoms, such as soft rot on the stalk, leaf wilting, and plant death. This study aimed to characterize the bacteria causing stalk rot disease in corn. Samples of infected plants were collected and identified morphologically, physiologically, and molecularly. The results showed that nine bacterial isolates were isolated from infected plants. All nine isolates showed positive hypersensitive responses on tobacco leaves. In comparison, only two bacterial isolates (BGO1 and BGO4) were positive on pathogenicity tests on corn. However, the BGO4 isolate caused the highest disease incidence with a faster incubation period. The BGO4 isolate was gram-negative with white-gray colored colonies. Physiological characterization of BGO4 also showed: positive catalase and indole, oxidase negative, fermentative oxidation, caused soft rot on potato, non-fluorescent, and sensitive to erythromycin. In addition, it can grow at 37-40°C and 5% NaCl, producing protease and lecithinase enzymes. The BGO4 also isolates infected rice, corn, sorghum, foxtail millet, celery, and *Aloe vera*. Morpho-physiology characteristics and diagnostic amplification of DNA by PCR using the *Dickeya*-specific primers (ADE1/ADE2) showed that the isolate belongs to the genus *Dickeya*. Further molecular characterization by analysis of the 16S rDNA using universal primer 27F/1497R successfully amplified the DNA band of BGO4 isolate measuring ±1300 bp. Phylogenetic analysis showed that it was in the same group as *Dickeya zeae* strain MS32 from Taiwan, strain DZ15SB01 (Thailand), and strain HNJF02 (China), with the coefficient of genetic distance ranging from 0.001 to 0.002. This study is the first report of *D. zeae* infecting corn in Gorontalo.

Keywords: Corn, *Dickeya zeae*, pathogen characterization, stalk rot

INTRODUCTION

Corn is one of the priority crops in Indonesia because it is used as food and feed. The challenge is the future strategy to meet the demand for corn as a feed, food, and energy raw materials. In addition, the increase in corn production in Indonesia is 5.21% per year (Panikkai et al. 2017), with the productivity in corn planting centers ranging around 5-9 t/ha. Therefore, the government continues to make various efforts to increase national corn production, especially in corn production centers such as East Java, South Sulawesi, Gorontalo, North Sumatra, and Lampung. For example, leading agro development in Gorontalo Province selects superior commodities in the corn processing industry in the food agriculture sector and coconut in the plantation sector as stated in the Regulation of the Minister of Industry No. 98/MIND/PER/8/2010 concerning Roadmap for Leading Industry Development of Gorontalo Province (Podungge et al. 2019). Another effort made by the Gorontalo government to increase corn farming is the establishment of cooperative institutions in corn production centers.

The development of corn farming is constrained by plant pest organisms that reduce the quality and quantity of production (Mirsam et al. 2021). So far, the major diseases of corn that have been reported as damaging in Indonesia are downy mildew, leaf blight, and leaf rust. However, recent infectious diseases have been observed that cause the plant to die completely, with symptoms of soft rot on the stalk that emit an unpleasant odor. This disease is generally found in the rainy season. According to Subedi et al. (2016), stalk decay after flowering is more prominent in reducing production than decay at the vegetative phase. Infected plants wilt with the yellowing of the leaves from the top; over time, the entire leaf turns yellow (Ahamad et al. 2015). External symptoms on the stalk include maceration and basal internodes. In addition, it causes soft rot and discoloration of infected tissue (Kumar et al. 2017a). In severe conditions, a foul odor occurs, and the plant collapses, so the potential for yield loss is quite significant. The disease is caused by the bacteria *Dickeya zeae*, formerly known as *Erwinia chrysanthemi* pv. *zeae* (Martinez-Cisneros et al. 2014; Ahamad et al. 2015; Subedi et al. 2016; Guan et al. 2020; Prokić et al. 2020). In

addition to infecting corn plants, *D. zeae* was also reported to infect several host plants throughout the world, including pineapple, taro corn, rice, banana, clivia, and sugarcane (Zhang et al. 2014; Hu et al. 2018; Zhang et al. 2018; Boluk et al. 2021; Yang et al. 2021). The existence of this pathogenic bacteria in Indonesia was previously reported by Aeny et al. (2020), infected pineapples in Lampung, Indonesia.

The *D. zeae's* ability to infect various dicotyledons and monocotyledons shows this bacterium has various virulence factors. *D. zeae* was reported as a pectolytic bacterium that produces pectic enzymes. These enzymes are important in plant tissue macerations, causing soft rot symptoms. However, several other factors also trigger the virulence and development of this bacterium, including the flow of irrigation water, fertilization with high nitrogen, and rainfall (Kumar et al. 2017a). Therefore, the aim of this study was to characterize the bacteria that cause corn stalk rot in Gorontalo Province, Indonesia.

MATERIALS AND METHODS

Exploration and isolation of the pathogen

A sampling of infected plants with corn stalk rot disease was conducted in Suka Makmur Village, Patilanggio Sub-district, Pohuwato District, Gorontalo Province, Indonesia. The sampling location was selected purposively, namely Pohuwato District, the highest maize-producing area in Gorontalo Province, Indonesia. Sampling was done by selecting maize based on specific criteria for symptoms of stalk rot disease caused by the bacterial infection (Figure 1). Symptomatic samples were taken and covered with tissue to retain moisture. The collected samples were then stored in separate plastic bags and immediately transported to the laboratory using a cooling box.

The stalk tissue between the diseased and healthy area was taken about 1 cm, surface sterilized using 75% ethanol for 30 seconds, 2% NaOCl for 1 minute, and rinsed with sterile distilled water. The sterilized sample was macerated using a 10% glycerol solution. The formed suspension was stored in an Eppendorf tube. Moreover, the suspension was cultured on Nutrient Agar (NA) media using the streak plate method. Then, a loop of suspension was carefully scratched in a quadrant pattern and then incubated at 30°C for 24 h. Finally, the dominant colony was selected and re-cultured on NA media to obtain a pure bacterial strain.

Preparation and measurement of the optical density of the bacterial suspension

The bacterial culture was re-cultured using Nutrient Broth (NB) media and incubated using a rotary shaker at 160 rpm for 24 hours. Bacteria growing on NB media were measured for their optical density using a spectrophotometer at a wavelength of 600 λ to obtain a cell concentration of 10^8 CFU/mL. Bacterial suspension with a 108 CFU/mL concentration was further analyzed on hypersensitive reaction, potato spoilage, pathogenicity, and host range tests.

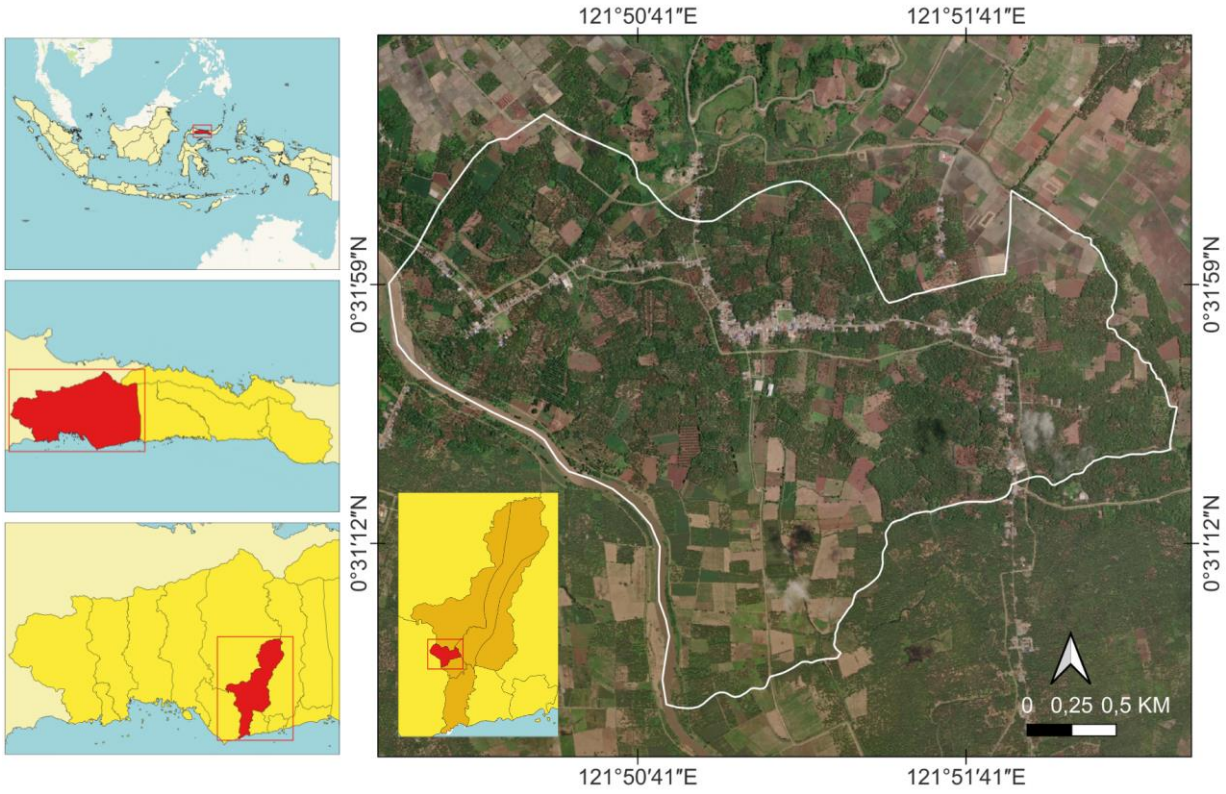

Figure 1. Sampling locations and disease symptoms found in Suka Makmur Village, Patilanggio Sub-district, Pahuwato District, Gorontalo Province, Indonesia

Hypersensitive reaction test

The hypersensitivity reaction test was carried out to determine the pathogenic and non-pathogenic bacterial isolates, according to Klement et al. (1990). A total of 1 mL of bacterial suspension with 10^8 CFU/mL concentration was injected underside the tobacco leaves. The necrosis symptoms were observed 24, 48, and 72 hours after injection.

Pathogenicity test on corn

The pathogenicity test on corn was carried out to confirm whether the bacterial isolates were pathogenic or non-pathogenic. The pathogenicity test was conducted in a greenhouse using polybags. The seeds of the NK7328 variety were treated with hot water at 55°C for 20 min, then airdried for 20 min on sterile tissue paper. The tested seeds were planted in polybags with sterile soil and combined media (1:1). The test was replicated three times. Corn was inoculated ten Days After Planting (DAP) following the method of Ahamad et al. (2015). Bacterial suspension with 10^8cfu/mL concentration was injected as much as 1 mL into the second segments from the base of the corn stalk. In addition to the negative control, corn plants were injected with sterile distilled water. The parameters observed were disease incidence and incubation period (when symptoms first appeared). The disease incidence rate was measured according to the following formula (Equation 1):

$$DI = \frac{A}{B} x\ 100\% \quad \text{..... (1)}$$

DI : Disease Incidence (%)

A : Number of plants infected with stalk rot

B : Number of plants inoculated for each bacterial isolate

Identification of the bacterial isolate based on morphological characteristics

Morphological characterization was carried out after obtaining pure isolates of tested bacteria. The tested bacterial isolate was identified based on the characteristics of the shape, elevation, margin, and colony color (Holt et al. 1994). Furthermore, the cell shape was observed using a compound microscope with a magnification of 1000x**.**

Identification of the bacterial isolate based on physiological characters

The physiological characterization of bacteria was carried out by several tests, including: the gram test using 3% KOH solution and bacterial gram staining (Mu'minah et al. 2015; Begum et al. 2017); the oxidase test used Sigma strip oxidase contains plastic strips with a paper zone saturated with a solution of N,N-dimethyl-1,4-phenylene diamine and alpha-naphthol, catalase test (Sudewi et al. 2020); oxidation fermentation test, tolerance test for temperature conditions of 37-40°C and acid using 5% NaCl, fried egg colony test on PDA media (Thakkar et al. 2016); sensitivity test to 15 µg erythromycin antibiotic (Kanzil et al. 2015), and fluorescent pigment production using King's B media (Nepali et al. 2018). In addition, enzyme activity tests were conducted on the ability of bacteria to produce enzymes: protease, lecithinase, and pectolytic enzymes, following the methods of Boluk et al. (2021), Thakur et al. (2021) and Kumvinit and Akarapisan (2019). In addition, protease and lecithinase enzyme activity tests were carried out using skim milk agar media and NA media enriched with 5% egg yolk emulsion. At the same time, the pectolytic enzyme test was carried out using Himedia Crystal Violet Pectate Medium. First, a well was made using a 3 mm cork borer on each test medium. Next, 50 µL of 24-hours-old bacterial suspension was added to each plate well and then incubated at 28°C for 24-48 hours. The formation of clear zones around the bacterial colonies observed the activities of protease and lecithinase enzymes.

The soft rot on potato tubers test was carried out according to the modified method of Azadmanesh et al. (2016). First, a sterile toothpick was dipped into a bacterial suspension with a concentration of 10^8 CFU/mL and incubated at 25°C for 24 hours. Then, toothpicks were injected into the potato tubers to a depth of 1 cm and placed in a sterile petri dish lined with wet cotton. Next, soft rot symptoms in potato tubers were observed 24, 48, and 72 hours after injection.

The ability of bacteria to degrade tryptophan to indole was conducted with Kovac's reagent, which contained para-dimethyl amino benzaldehyde in a test tube. The formation of a red ring layer on the surface of the bacterial suspension identified a positive indole test.

Host range test

The host range test was carried out on seven tested plant species following the method of Aeny et al. (2020). The tested plants were rice, sorghum, wheat, foxtail millet, *Aloe vera*, celery, and corn. Next, a 48-hour-old bacterial suspension with a 10^8 CFU/mL concentration was injected into the stem. Disease incidence was observed every day for five days and calculated using equation 1. The test was conducted in a completely randomized design consisting of seven host plants as the treatment and repeated three times.

Identification of bacterial isolate based on the polymerase chain reaction

DNA extraction

DNA extraction was performed using ZymoBiomics Quick-DNA™ Fungal/Bacterial Miniprep Kit. The working principle of the kit was to add 50-100 mg bacterial cells suspended in water to a ZR BashingBead™ Lysis Tube. The ZymoBIOMICS lysis system was achieved by bead beating with the innovative ultra-high-density BashingBeads. The DNA was then isolated and purified using Zymo-Spin™ Technology.

Amplification of bacterial DNA

Amplification using specific primers was done to confirm the tested bacteria's genus before sequencing the 16S rDNA. The specific primers used were ADE1 (5'-GATCAGAAAGCCCGCAGCCAGAT-3') and ADE2 (5'-CTGTGGCCGATCAGGATGGTTTTGTCGTGC-3') with ±420 bp target amplicon. Furthermore, to determine species, the partial 16S rDNA gene fragment was amplified using the universal primers 27F (5-

AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-GGTTACCTTGTTACGACTT-3) with ±1500 bp target amplicon. PCR was performed using a SensoQuest Thermal Cycler (Germany) machine with the following program: 1 cycle of initial denaturation at 94°C for 2 minutes, 35 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 62°C for 45 seconds, extension at 72°C for 2 minutes, and final extension at 72°C for 3 minutes (ADE1/ADE2 primers); 1 initial denaturation cycle at 95°C for 3 minutes, 35 cycles consisting of denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds, primer extension at 72°C for 1 minute, and the final extension at 72°C for 3 minutes (27F/1492R primers). Bacterial DNA was electrophoresed at a voltage of 110 V for 50 minutes. The electrophoresis results were visualized with a UV transilluminator. While the photos were taken using the camera.

Analysis of sequencing of the 16S rDNA

The positive amplicon from the universal primer was sent to FirstBase (Malaysia) for sequencing. First, the sequence result was analyzed using the basic local alignment search tool (BLASTN 2.13.0+) with an optimization program to obtain the sequence of DNA bases contained in the National Center for Biotechnology Information (NCBI) site. Next, the nucleotide sequence results were analyzed using multiple alignments Clustal on the software Bioedit Sequence Alignment Editor 7.2 version. Finally, the kinship relationship between isolates was constructed using software Molecular Evolutionary Genetic Analysis Software 10.0 version (MEGAX) with bootstrap 1000 times repetitions.

Data analysis

Data analysis was carried out at the stage of testing the host range. Observational data were analyzed statistically with a One-way Analysis Of Variance (ANOVA). If there was a treatment effect, then proceed by Least Significant Difference (LSD) at the 5% significance level (0.05)

RESULTS AND DISCUSSION

Symptoms of corn stalk rot

Symptoms of corn disease were soft and slimy rot on the stalk, wilted plant, yellowing of leaves, and an unpleasant odor from the infected part of the stalk. Infected corn showed death, but some only showed symptoms of wilted leaves; first to second rootstock segments turned into a wet brown color with clear boundaries. It was also observed that the disease became more serious during the rainy season, which reduced the productivity of maize. A total of nine isolates were isolated from the infected samples, namely BGO1, BGO2, BGO3, BGO4, BGO5, BGO6, BGO7, BGO8, and BGO9.

Pathogenicity characteristics of bacterial isolates

All nine bacterial isolates showed a positive hypersensitive response 24-48 hours after inoculation. The symptoms of tobacco leaf damage were clearly observed in the inoculated leaf area in the form of yellow necrosis. Meanwhile, the pathogenicity test showed that only two BGO1 and BGO4 isolates caused stalk rot on corn. However, the BGO1 isolate suspension caused brown color discoloration, dryness, and death ten days after inoculation. Therefore, it can be suspected that the death of maize inoculated with BGO1 was not caused by bacterial infection; other factors, such as microbial infection by the fungus, could cause it. Isolate BGO4 had a shorter disease incubation period than other isolates, ranging from 1 to 4 Days After Inoculation (DAI), with a disease incidence of up to 83.33% (Table 1).

Pathogenicity test results showed wilting in lower leaves, and infected corn stalk tissue became brown and softer than healthy tissue (Figure 2). The damage to tissue structure was caused by the activity of bacterial pectolytic enzymes, which damage the binding material of plant cells. Nevertheless, symptoms persisted, and some tested plants died at 3 DAI. Rotting of stem tissue was a symptom of death in infected corn, while the plants inoculated with sterile distilled water did not show any symptoms of damage.

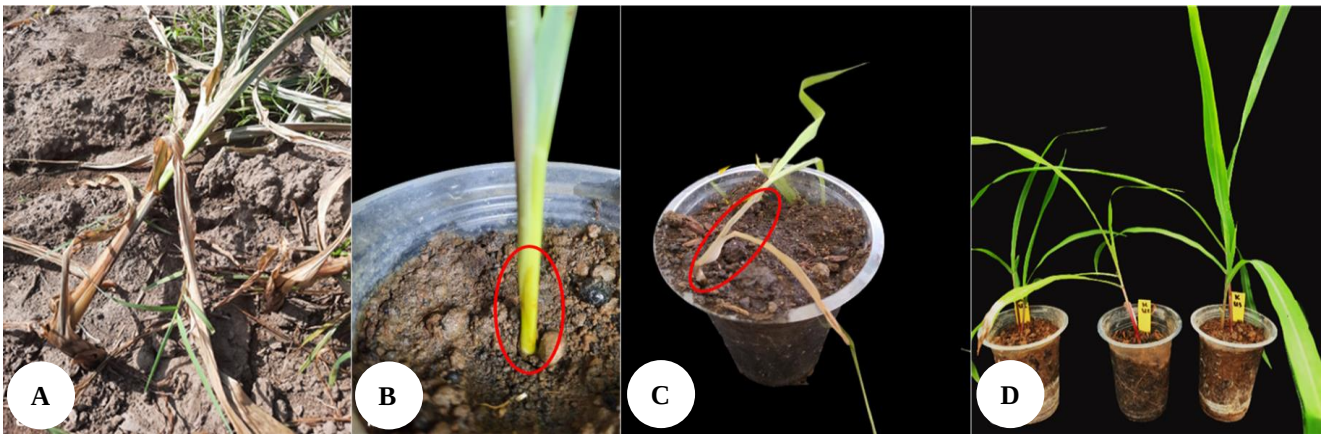

Figure 2. Corn plants infected with stalk rot disease in the crop (A); Necrotic symptoms develop around the point of inoculation (B); Symptoms of plant death 3-6 days after inoculation (C); Plants that remain healthy in the control treatment (D)

Morphological characters of BGO4 isolate

The re-isolation of BGO4 isolate from the pathogenicity test was then identified based on morphological characteristics. On NA medium, BGO4 isolate had white-gray colored colonies with round shape, elevation convex, and entire margin. At 2 to 3 days after incubation, the colony shape turned nearly round to an irregular margin. On Kings B media, bacterial colonies were a white-cream color, shiny, round, and convex shapes, and did not produce fluorescent pigment. In addition, the bacterial cells were bacilli with rounded ends. Based on the morphological characteristics, the BGO4 isolate was suspected of belonging to the genus *Dickeya*.

Physiological characteristics of BGO4 isolate

Physiological characteristics of the BGO4 isolate showed catalase positive, indole positive, and oxidase negative and caused soft rot on potato tubers. In addition, isolate BGO4 was oxidative fermentative by changing the color of OF medium to yellow, both covered and uncovered. The bacteria formed colonies like fried eggs on PDA media and were able to grow under relatively high-temperature conditions at 37-40°C and 5% NaCl media (Table 2).

The sensitivity to antibiotics showed that the BGO4 isolate was sensitive to erythromycin 15µg. A positive result was indicated by forming a clear zone around the antibiotic paper disc. The bacterial growth colonies on media without antibiotics were evenly distributed on the surface (Figure 3.A). Another physiological characteristic tested in this study was the activity of protease and lecithinase enzymes. Isolate BGO4 could grow on the skim milk agar media and NA media, enriched with 5% egg yolk emulsion, forming clear halo zones (Figure 3.B). This showed that bacteria could produce protease and lecithinase enzymes.

The production of pectolytic enzyme was indicated by the ability of bacteria to form clear zones on CVP media 72 hours after incubation (Figure 3.C). The clear zone formation indicated that the BGO4 isolate could degrade sodium poly pectate in the media. In addition, the pectolytic activity of bacteria was capable of rotting potato tubers. The indole test showed a direct red color change in the BGO4 isolate surface when it was dropped with Kovac's reagent, indicating the formation of indole (Figure 3.D).

Host range of BGO4 isolate

In the host range test, maize and celery showed soft rot symptoms at 1 DAI with 4.76% and 3.33% disease incidence, respectively. Meanwhile, the other five tested plants showed stalk rot symptoms at 2 DAI to 4 DAI, except wheat, which showed no symptoms till the end of observation. Although the initial infection in *Aloe vera* was found at 2 DAI and increased to 5 DAI with 73.33% disease incidence, this was not significantly different from the disease in celery and corn. The initial symptom in corn, sorghum, and foxtail millet was necrosis that developed around the inoculation site. These symptoms indicated stem tissue decay which causes plants to wilt and die. Meanwhile, the symptoms of infection in celery were leaf drooping and wilting (Figure 4).

Molecular characters of BGO4 isolate

The isolated bacteria were then confirmed by DNA amplification using specific primers for the genus *Dickeya*, i.e., ADE1/ADE2. The ADE1/ADE2 primers amplified DNA bands of the BGO4 isolate measuring at ± 420 bp (Figure 5.A). Furthermore, the DNA of isolate BGO4 was amplified again using primer 27F/1492R, the amplicon of which was used for sequencing analysis. Finally, using universal primer pairs 27F/1492R successfully amplified a DNA band of BGO4 isolate measuring at ±1300 bp (Figure 5.B).

Table 1. Hypersensitive reaction and pathogenicity test on corn of 9 bacterial isolates

Isolates	Hypersensitive reaction at (HAI)			Pathogenicity test on corn	
	24	48	72	DI (%)	IP (Day)
BGO1	(-)	(+)	(+)	16.67	10
BGO2	(+)	(+)	(+)	0	0
BGO3	(-)	(+)	(+)	0	0
BGO4	(+)	(+)	(+)	83.33	1 s/d 4
BGO5	(-)	(+)	(+)	0	0
BGO6	(+)	(+)	(+)	0	0
BGO7	(+)	(+)	(+)	0	0
BGO8	(-)	(+)	(+)	0	0
BGO9	(-)	(+)	(+)	0	0
Control	(-)	(-)	(-)	0	0

Note: (+): symptoms of necrosis and decay; (-): no signs of damage; DI: Disease Incidence; IP: Incubation Period (day); HAI: Hours After Inoculation

Table 2. Physiological characterization of BGO4 bacterial isolate from Gorontalo Province and reference *Dickeya zeae*

Characters	BGO4 isolate	*Dickeya zeae**
Gram reaction	-	-
Hypersensitive on tobacco	+	+
Oxidase	-	-
Catalase	+	+
Lecithinase activity	+	+
Protease	+	nd
Potato soft rot	+	+
Fluorescence on King B medium	-	-
Glucose metabolism	O/F	O/F
Grows at 37°C	+	+
Growth at NaCl 5%	+	v
Erythromycin sensitivity 15µg	+	nd
"Fried egg" like colonies on PD medium	+	+
Indole test	+	nd
Pathogenicity assay on corn	+	+

Note: * Prokić et al. (2020); (+): indicates positive; (-): indicated negative; v: indicated variable reaction; OF: Oxidative-Fermentative; nd: no data

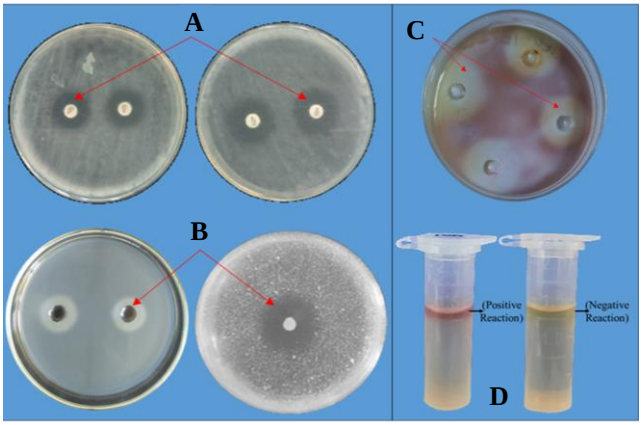

Figure 3. A. The formation of a clear zone indicated the sensitivity of the isolate to 15 g antibiotics after 24 hours of incubation. B. The activity of the lecithinase and protease enzymes was indicated by the formation of a clear zone. C. The pectolytic activity of bacteria on CVP media. D. The reaction of bacteria on indole test

Homology levels of BGO4 isolate DNA sequences with bacterial strains on the GeneBank

The results of the DNA sequence analysis of the BGO4 isolate based on the amplification of the 16S rDNA using BLASTN 2.13.0+ program showed a different sequence identity with each bacterial strain in GenBank. The sequence length ranges of BGO4 isolate with similar bacterial strains in GenBank ranged from 1346-504 bp. In addition, coverage query from BLASTN results also showed a very high percentage of the length of DNA sequence conformity between isolate BG04 and similar bacterial strains in GeneBank of ≥99% (Table 3).

Analysis of the BLASTN 2.13.0+ program showed that the DNA sequence of isolate BGO4 had the highest similarity with *D. zeae* (> 90% with an e value of 0.0). Isolate BGO4 had similarities to *D. zeae* strain DZ15SB01, HNJF02, MS32, MAFF311098, DZ-B1-1, DzM2, VR01P1 and *E. chrysanthemi* strains ECH279, ICMP4649, and A5264 with homology values ranging from 98.20%-99.20%. In addition, isolate BGO4 had a relatively far level of similarity with the outgroup comparison isolate from the *Pseudomonas aeruginosa* strain KSG bacteria group with a homology value of 84.90% (Table 4).

Phylogenetics of BGO4 isolate based on nucleotide base sequence

Phylogenetic analysis showed that isolate BGO4 was in the same group as *D. zeae* strain MS32 from Taiwan, strain DZ15SB01 from Thailand, and strain HNJF02 from China, with genetic distance coefficient values ranging from 0.001 to 0.002. In addition, isolate BGO4 also had a close kinship with bacteria *D. zeae* strain DzM2 from India, strain VR01P1 from Australia, strain MAFF311098 from Japan, and bacteria *E. chrysanthemi* strain A5264 from the USA, strain ECH279 from Malaysia, and strain ICMP4649 from New Zealand, with a fairly close genetic distance coefficient of 0.003. In contrast, isolate BGO4 had a fairly distant relationship with the KSG outgroup comparison strain from the *P. aeruginosa* bacteria group with a genetic distance coefficient of 0.085 (Figure 6).

Analysis of genetic diversity of BGO4 isolate with bacterial strains on GenBank

The genetic diversity of molecular-based bacterial specimens was further analyzed using the DNA sequence alignment method between BGO4 and bacterial specimens deposited at NCBI. The alignment of DNA sequences showed a very high genetic variation between bacterial strains. For example, the alignment of the DNA sequences of the BGO4 isolate with the bacterial strains in GenBank showed that the amplified 16S rDNA was generally in a region with high and stable conservation. In addition, there was also very high genetic variation in the nucleotide sequence or loci of BGO4 isolate155, 267, 275, 277, 280, 336, 353, 355, 357-359, 366-369, 371-373, 778, 786, 828, 834, 901, 905-907, 917, 920-922, 935, 937, 962, 970, 1034, 1036-1038, 1244, and 128 (Figures 7 and 8).

Table 3. Information on the similarity of BGO4 isolate with DNA sequences in the GeneBank NCBI

Organisms	Strain codes	No. accession	Sequence length (bp)	Query cover (%)	Year	Origin
Dickeya zeae	DZ15SB01	KY817902	1503	99.00	2017	Thailand
Dickeya zeae	HNJF02	KY784647	1504	99.00	2017	China
Dickeya zeae	MS32	OM095445	1408	100.00	2022	Taiwan
Dickeya zeae	MAFF311098	AB713546	1370	99.00	2014	Japan
Dickeya zeae	DZ-B1-1	KJ438949	1414	99.00	2014	Mexico
Dickeya zeae	DzM2	MT037020	1365	99.00	2020	India
Dickeya zeae	VR01P1	MT995003	1400	99.00	2021	Australia
Erwinia chrysanthemi	ECH279	KF058032	1399	99.00	2013	Malaysia
Erwinia chrysanthemi	ICMP4649	EF530560	1346	99.00	2007	New Zealand
Erwinia chrysanthemi	A5264	EU821583	1358	99.00	2008	USA

Table 4. Matrix of DNA sequence identity for BGO4 isolate paired with isolates in GenBank

Codes	BGO4	A	B	C	D	E	F	G	H	I	J
BGO4	ID										
A	0.992	ID									
B	0.992	1	ID								
C	0.990	0.990	0.990	ID							
D	0.987	0.990	0.990	0.984	ID						
E	0.984	0.990	0.990	0.982	0.993	ID					
F	0.986	0.992	0.992	0.982	0.995	0.996	ID				
G	0.983	0.989	0.989	0.980	0.993	0.996	0.997	ID			
H	0.984	0.987	0.987	0.981	0.996	0.990	0.992	0.989	ID		
I	0.982	0.988	0.988	0.980	0.991	0.996	0.994	0.993	0.993	ID	
J	0.849	0.850	0.850	0.852	0.851	0.853	0.850	0.850	0.849	0.851	ID

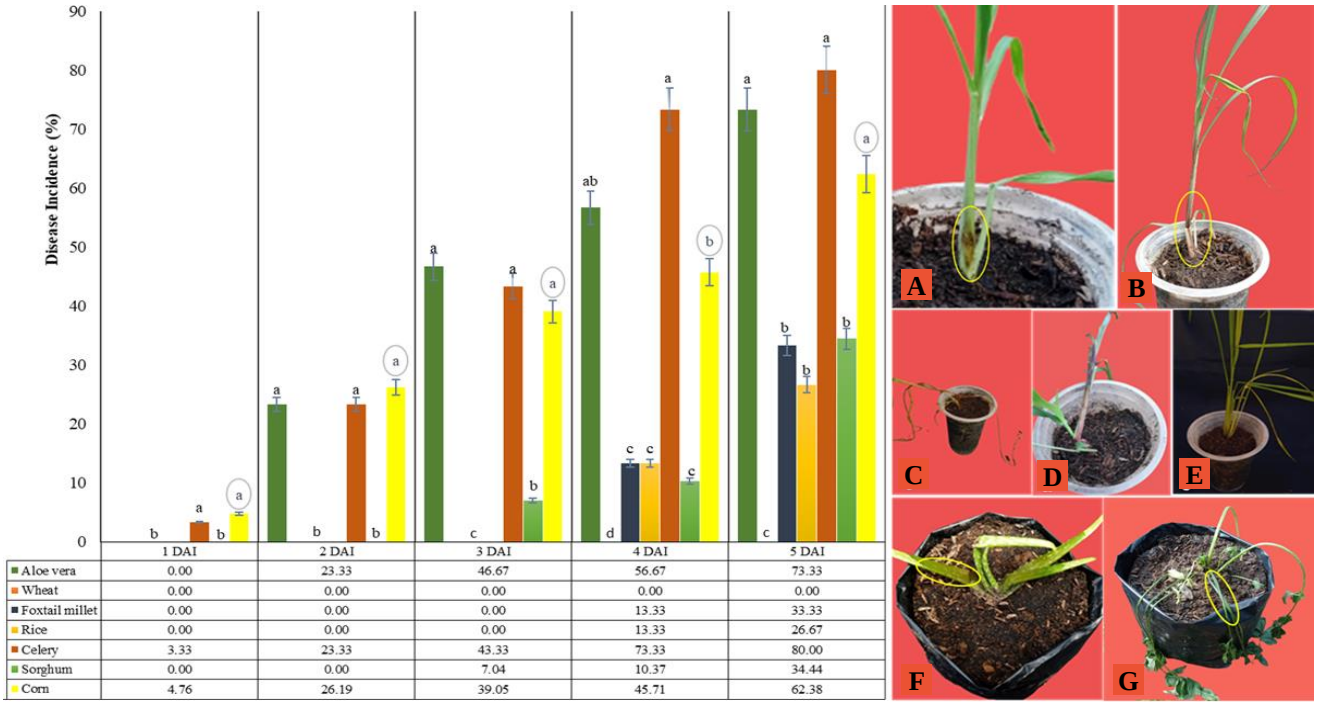

Figure 4. *Left*: The incidence of stalk rot disease from several plant species after inoculation with the BGO4 isolates; *Right*: Symptoms of necrosis in sorghum and foxtail millet (A-B); stalk rot symptoms in sorghum, foxtail millet, and rice after 2-5 DAI (C-E); soft rot on *Aloe vera* (F) and celery (G)

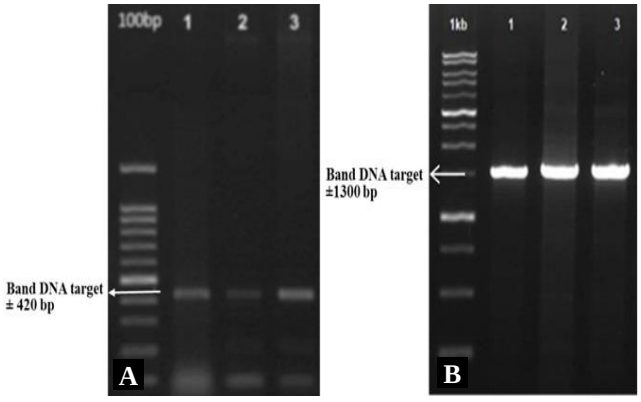

Figure 5. Visualization of DNA fragments from gene pelADE amplification with primers ADE1/ADE2 (a) and 16S ribosomal DNA amplification (b) of isolate BGO4 by Polymerase Chain Reaction (PCR) method. 1kb: Marker/DNA ladder; 1, 2, and 3, replications

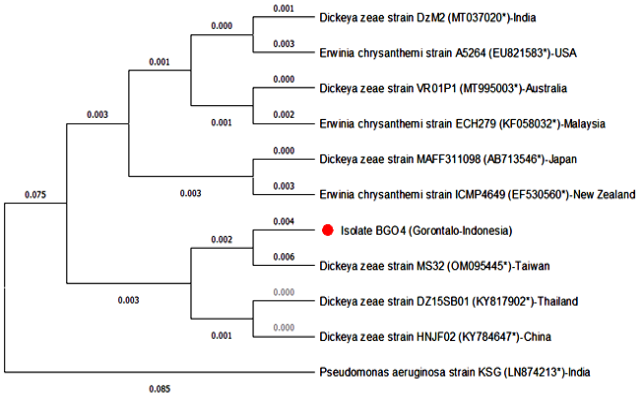

Figure 6. Phylogenetic tree of isolate BGO4 based on analysis of Neighbor-Joining Tree with Kimura 2-parameter model with distance matrix calculation of genetic bootstrap replications 1000 implemented in Bioedit 7.2 program and MEGAX. (●) Research isolate; (*) Accession number

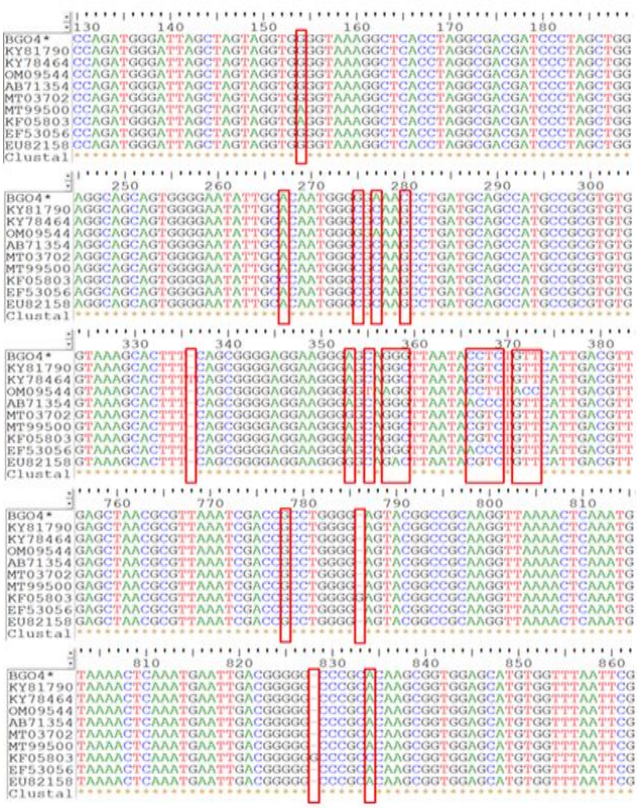

Figure 7. Alignment of nucleotide bases of isolate BGO4 with several bacterial strains in GeneBank at loci 130 to 862. The *(asterisk) indicates identical and highly conserved nucleotide bases; red boxes indicate areas of high genetic variation

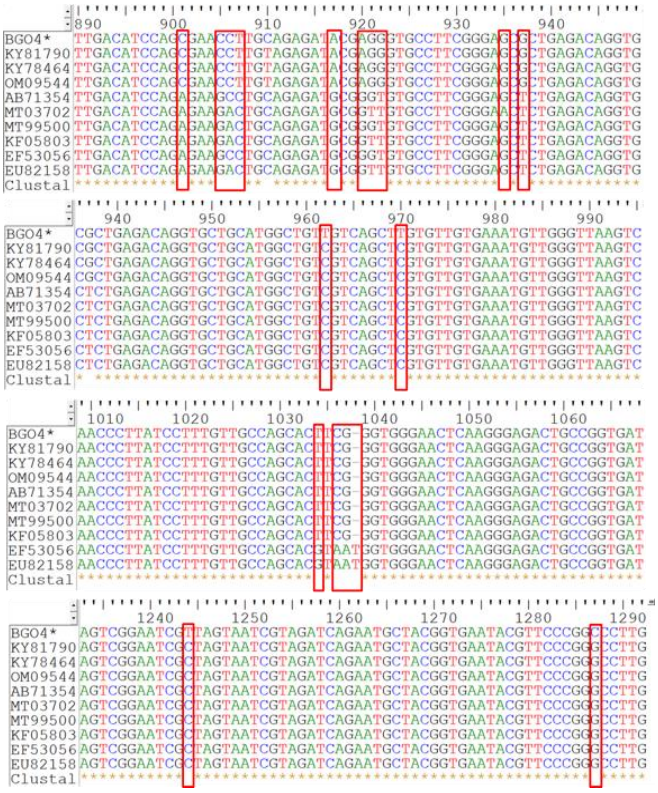

Figure 8. Alignment of nucleotide bases of isolate BGO4 with several bacterial strains on GeneBank at loci 890 to 1292. The *(asterisk) indicates identical and highly conserved nucleotide bases; red boxes indicate areas of high genetic variation

Discussion

The early symptoms of bacterial stalk rot disease in corn are wilting, softening, and rotted stalk tissues. During the survey in Gorontalo, the disease was in its early generative phase. In addition, symptoms of ear rot and drooping were found before physiologically ripe. The stalk rot disease caused by *D. zeae* bacteria plagues corn crops worldwide (Ahamad et al. 2015; Subedi et al. 2016; Jittikornkul et al. 2017; Kumar et al. 2017b; Prokić et al. 2020). Ahamad et al. (2015) reported that *D. zeae* infected hybrid, composite and local corn in India with different disease incidence rates in each region, ranging from 10-39%.

Two of the nine bacterial isolates found in this study were capable of causing stalk rot disease, based on the pathogenicity test on corn. For example, isolate BGO4 could damage stems one day after inoculation. The same condition was reported by Kumar et al. (2015) stated the internodes of corn plants were damaged after 24 HAI of *D. zeae*, and the virulence of bacteria caused death in sweet corn plants at 4-5 DAP. Jittikornkul et al. (2017) also reported that *D. zeae* isolated from corn was able to cause stalk rot disease in corn 2 DAI. Kumar et al. (2017b) reported that pectolytic enzymes play an important role in the degradation of plant tissue.

The BGO4 isolate from Gorontalo was identified as *D. zeae* based on the characteristics of its colony growth. Previous studies reported that *D. zeae* colonies were circular and convex. While creamish-color colonies on King's B media and fried eggs colonies on PDA or NA media enriched with 2% glucose (Alic et al. 2017; Zhang et al. 2020; Mokrani and Nabti 2021). Prokić et al. (2020) reported that *Dickeya* colonies isolate from corn and were grown on NA media for 2-3 days with the characteristics of a grayish-white, shiny, non-mucous, round-shaped colony with irregular boundaries. In addition, the bacterium could also degrade potato tissue by softening the tissue at the inoculation site, and the presence of a bad smell indicates positive pectolytic activity. Muturi et al. (2018) stated that *Pectobacterium* and *Dickeya* are pectolytic bacteria widely reported to cause soft rot disease. One distinguishing characteristic of the genus *Dickeya* and *Pectobacterium* is the ability of *Dickeya* to produce indole. The genus *Pectobacterium* lacks the tryptophanase enzyme that breaks down the amino acid tryptophan into indole, so the indole test is negative (Kamau 2020). However, these two genera of bacteria have some characteristics in common, including: catalase activity, negative oxidase, the ability to utilize carbohydrates both fermentatively and oxidatively, and the production of the enzyme lecithinase (Czajkowski et al. 2015; Pratama et al. 2022). The BGO4 isolate can produce protease, and lecithinase enzymes play a role in the decomposition of plant cell walls or the maceration of plant tissues (Boluk et al. 2021). The characteristics of isolate BGO4 are similar to those of *D. zeae* isolated from maize stalk rot strains in Serbia (Prokić et al. 2020).

The BGO4 isolated from corn plants could infect five other tested plants. Therefore, rice, sorghum, barley, celery, and *Aloe vera* can potentially host *D. zeae* in Indonesia. The ability to infect several tested plants strengthens the suspicion that BGO4 isolate is a bacterium of the genus *Dickeya* which was previously reported to have many host plants (Hu et al. 2018; Li et al. 2020). Aeny et al. (2020) reported that *D. zeae* isolate from pineapple plants caused soft rot disease in lettuce, chicory, celery, dragon fruit, corn, leeks, tomatoes, green beans, long beans, *Aloe vera*, guava, pak-choy, chrysanthemums, and orchids. In Japan, *D. zeae* was isolated from maize, rice, calanthe orchids, and Setaria grass (Suharjo et al. 2014).

Morpho-physiology characteristics and diagnostic amplification DNA by PCR using the Dickeya-specific primers (ADE1/ADE2) showed the isolate belongs to the genus *Dickeya*. The species identity of this bacterium was confirmed using the partial sequence analysis of the 16S rDNA gene. The universal primer pair 27F/1492R detected the presence of the 16S-rDNA encoding gene in BGO4 isolates, where this gene is known to be the skeleton of the ribosome which is generally used to identify bacterial groups, highly conserved region, and plays an important role in protein synthesis (Jayaseelan et al. 2018). Furthermore, this gene is known to be a ribosomal framework generally used to identify all groups of bacteria. The 16S rDNA sequences are widely used to identify phylogenetic relationships between known and unknown bacteria (Beissner et al. 2012; Braun et al. 2021; Jones et al. 2021; Mirsam et al. 2022; Shah et al. 2022). Phylogenetic analysis of BGO4 isolates showed that this bacterium belongs to the *D. zeae* group with the highest similarity to the *D. zeae* strain DZ15SB01 from Thailand and the HNJF02 strain from China.

The alignment of the BGO4 DNA sequences with the bacterial strains in GeneBank showed very high genetic variation in the nucleotide sequence or loci. In addition, Zhang et al. (2022) reported the sequencing genomes of three *D. zeae* strains isolated from banana plants showed phenotypic diversity in antibiotic effects, colony pigments, and virulence levels between strains. Therefore, studies of the genetic diversity of bacterial strains need to be carried out to suppress the spread of new virulent strains.

In conclusion, it was observed that out of nine isolates, only BGO4 isolate was found to cause stalk rot disease in corn plants. Further molecular characterization with 16S rDNA analysis revealed that the isolate was identified as *D. zeae*. The isolate BGO4 was also able to infect six plant species that could be potential hosts for this bacterium in Indonesia. This study is the first report of *D. zeae* infecting corn in Gorontalo. This is preliminary information to prevent the spread of *Dickeya* bacteria in Gorontalo Province from making a control decision and reducing farmers' losses. Identifying this bacteria is important for determining the characteristics of the bacteria that can be used as a reference in control technology, such as the sensitivity of bacteria to the antibiotic erythromycin or biological agents that produce appropriate antibiotic compounds in controlling *D. zeae*. In addition, information on the host range of this bacterium can be used as a reference for agricultural and horticultural officers to anticipate this bacterial attack.

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New corn resistant lines to stalk rot disease (*Dickeya zeae*) in Indonesia

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Abstract. *Suriani, Patandjengi B, Muis A, Junaid M, Mirsam H, Azrai M, Efendi R, Sebayang A. 2023. New corn resistant lines to stalk rot disease (*Dickeya zeae*) in Indonesia. Biodiveritas 24: 3190-3200.* Stalk rot disease caused by *Dickeya zeae* is one of the important diseases of corn in Indonesia. Host resistance cultivars are an effective and sustainable control measure of the disease. Therefore, the present study aimed to evaluate the resistance of 15 S1 hybrid maize lines to stalk rot disease. The research was conducted in two seasons (DS and WS) using a randomized block design with 3 replications. The *D. zeae* suspension with 10^8cfu/mL concentration was inoculated into the plant test 45 Days After Planting (DAP). Disease incidence and severity were observed during the two seasons. The results showed that all tested lines were infected with stalk rot disease but had various resistance reactions. Disease incidence and severity in the dry season were higher than in the rainy season. In the rainy season all test lines followed the 3 models of disease development, but in the dry season, all lines followed the monomolecular model. Further analysis showed that 3 lines of hybrid maize had the lowest AUDPC value with a protection index of more than 50% in two growing seasons. Stalk lignin content had negative correlation with a disease incidence of -0.60877, so it can be used as a parameter of plant resistance to disease. Tested lines that show resistance to the disease could potentially be useful as new varieties of maize.

Keywords: Corn lines, *Dickeya zeae*, resistance, screening, stalk rot

INTRODUCTION

Stalk rot disease caused by *Dickeya zeae* has been reported in infected corn in several regions of the world (Martinez-Cisneros et al. 2014; Ahamad et al. 2015; Subedi et al. al. 2016; Guan et al. 2020; Prokić et al. 2020; Suriani et al. 2023). This bacterium can survive up to temperatures of 50°C, and it can cause disease in tropical and sub-tropical regions (Adesh et al. 2017; Yildiz and Aysan 2022). The decrease in corn production due to this disease is caused by the decay of stalk tissue, which cuts off the flow of nutrients to plant parts (Adesh et al. 2017). Stalk rot disease in India was reported to infect hybrid, composite, and local varieties of corn with a disease incidence of up to 34.80% (Ahamad et al. 2015; Kumar et al. 2016). In Indonesia, *D. zeae* has been found to be associated with corn infection in Lampung, Central Java, Gorontalo, and West Sulawesi (Anonymous 2019; Aminah 2020). Therefore, disease control efforts are needed to reduce yield loss.

The current control method for stalk rot in corn still favors synthetic pesticides. However, this requires substantial costs, and chemical residues are harmful to environmental sustainability (Odelade and Babalola 2019; Suriani et al. 2021; Kanaan 2021; Mirsam et al. 2021a). In addition, the use of pesticides can trigger pathogen gene mutations to become resistant, so these strains are difficult to control (Hobbelen et al. 2014). Efforts to control plant diseases can basically be carried out by suppressing the development of pathogens through assembling resistant varieties, planting pathogen-free seeds, and using pesticides (O'Brien 2017). Thus, pathogen infections can be suppressed below the economic threshold value. The use of resistant varieties is one of the most widely used options because this method is more effective, safe, and relatively inexpensive (Maheshwari et al. 2020; Sikirou et al. 2021; Wang and Dong 2021). Assembling disease-resistant varieties can be developed either through selection/screening or crossing (Fetene et al. 2020). If resistant genotypes can be identified before the reproductive growth stage, this can help breeders accelerate the development of resistant varieties (Viriyasuthee et al. 2019).

The availability of maize varieties that are resistant to bacterial stalk rot has not been reported in Indonesia. This is because only three main diseases of maize have been reported in Indonesia, namely downy mildew, leaf blight, and leaf rust, so variety descriptions generally include the level of resistance to these three diseases (Mirsam et al. 2021b). Therefore, it is necessary to screen the population

or lines of maize for bacterial stalk rot disease to develop resistant varieties. Plant disease resistance testing should be carried out under endemic conditions. Environmental factors must be suitable for the development of pathogen disease cycle, such as spore release and dissemination. The development of stalk rot disease in corn plants is influenced by environmental factors, such as temperature, low oxygen concentrations, and the availability of free water in the field (Reverchon and Nasser 2013). Disease epidemics can be created by inoculating the pathogen of the test plant or the inoculum source plant that was planted prior to planting the test material. Inoculation of plant pathogens can be carried out through planting in infected soil plots for soil-borne pathogens, spraying pathogen suspension for air-borne pathogens, and soaking the seeds with the pathogen suspension for seed-borne diseases (UnNabi and Choudhary 2015).

Based on this, the present study was conducted to evaluate 15 S1 and parental lines of hybrid maize in the rainy season (WS) and dry season (DS).

MATERIALS AND METHODS

Study site

The research was conducted at the Bajeng Agricultural Research and Development Installation (IP2TP), Gowa District, South Sulawesi, Indonesia from February to September 2022. The research was arranged in a Randomized Block Design (RBD) which was repeated three times. The genetic material used was 15 S1 of high-yielding hybrid corn lines and two control lines, which were hybrid maize parents (Table 1). The research was carried out in two seasons, namely rainy season (February-May 2022) and dry season (June-September 2022).

Planting of test materials

The test genotypes were planted in two rows 5 m long with a spacing of 70 x 20 cm. One seed per hole was planted, so the population of each genotype per replicate was ± 50 plants. Fertilizers were applied twice, first time at 10 days after planting (HST) using Urea and NPK fertilizers at a dose of 150 kg/ha and 400 kg/ha, respectively. The second application at 30 HST using Urea fertilizer, a dose of 150 kg/ha. Plant maintenance was performed until 90 DAP by irrigation and weed control according to the condition of the plants.

Table 1. List of test materials consisting of 15 S1 lines of high-yielding potential corn and 2 control lines

Genotypes		
MTD1-1	MTD2-2	MTD5-2
MTD1-4	MTD3-5	MTD5-3
MTD1-5	MTD3-7	MTD6-2
MTD1-6	MTD4-2	Mal 03
MTD1-7	MTD4-4	MGOLD
MTD2-1	MTD5-1	

Preparation of *Dickeya zeae* bacterial isolate and pathogen inoculation

Isolates of *D. zeae* were taken from the Research Center for Food Crops, Research Organization collection. Bacterial isolates were propagated in Nutrient Broth media and shaken for 24 hours. Furthermore, turbidity level of the bacterial suspension was measured using a spectrophotometer at a wavelength of 600 nm to obtain an Optical Density (OD) value of ±0.862 which was equivalent to a bacterial concentration of 10^8 cfu/mL. A 1 mL of bacterial suspension was injected into pre-silking plants or 45 days after planting (Ahamad et al. 2015). The bacterial suspension was injected into the second stalk segment from the soil.

Observation of incidence and severity of corn stalk rot disease

Disease incidence was observed every week as much as 6 times. The first observation was carried out one Week After Inoculation (WAI) by counting the number of plants infected with stalk rot disease. Observations were accumulated using the following formula (Equation 1):

$$DI = \frac{A}{B} x\ 100\% \quad \text{.....} \quad (1)$$

Where:
DI : disease incidence (%),
A : number of plants infected with stalk rot, and
B : number of plants observed in each line

Observation of disease severity was carried out when the plants were 90 HST by dividing the stalks of 10 plant samples per unit and measuring the severity of the disease by giving a score based on Directorate of Maize Research India (2012) presented in Table 2.

Table 2. The rating scale for measuring disease severity to stalk rot disease on corn (Directorate of Maize Research India 2012)

Scale	Description
1	The infection is limited to a very small spot in the pith at the site of inoculation.
2	Disease infection spreads in half of the length of the inoculated internode in the pith and critical tissues, rind not infected
3	Infection covers the entire length of the inoculated internode but does not cross the nodal plates. The rind is green and the symptoms are not visible extremely, but plant shows sign of wilting.
4	The infection spreads to another adjacent internode. The pith and critical tissues are degenerated. The rind of the inoculated internode is affected and the plant wilts.
5	The diseases spreads in the three or more internodes. The pith, cortical tissue, and vascular are rotten and disorganize. Rind discoloured, plant wilt and may topple down finally.

Table 3. Criteria for the resistance of maize genotype to stalk rot infection

Disease Incidence	Resistance Criteria
< 10%	Resistant (R)
>10.1-25%	Moderately resistant (MR)
>25.1-50%	Moderately susceptible (MS)
>50%	Susceptible (S)

The disease scale was then transformed into the disease severity percentage formula as follows (Equation 2):

$$DS = \frac{\sum(n \times v)}{Z \times N} \times 100\% \quad \text{..... (2)}$$

Where:
DS : disease severity
N : number of infected plants in each category;
V : scale value on each affected plant;
Z : highest scale value;
N : number of plants observed in each treatment.

The resistance categories of the test genotypes to stalk rot disease (Table 3) were determined by Hooda et al. (2018).

Analysis of disease progression models and infection rates

Model analysis of stalk rot disease development was carried out based on model accuracy tests or goodness-of-fit tests for disease development in the three most widely used models, namely monomolecular, logistic, and Gompertz (Xu 2006). The selection of model through the transformation of data on the proportion of disease (x) that had been collected, into ln (1/(1-x) for monomolecular models, ln{x/(1-x)} for logistic models, and {-ln (-ln x)} for the Gompertz model. This new data is regressed linearly with respect to the time (t) of the development of the disease. The calculation of infection rate was based on the results of model selection disease progression using the following formula:

Monomolecular model:

$$r_m = \frac{1}{t}\left(\ln \frac{1}{1-x_t} - \ln \frac{1}{1-x_0}\right) \text{ per time unit} \quad \text{..... (3)}$$

Logistic model:

$$r_l = \frac{1}{t}\left(\ln \frac{x_t}{1-x_t} - \ln \frac{x_0}{1-x_0}\right) \text{ per time unit} \quad \text{..... (4)}$$

Gompertz model:

$$r_g = \frac{1}{t} - \ln\{-\ln(X_t)\} + \ln(-\ln(X_0)\} \text{ per time unit} \quad (5)$$

Where:
x_t : the proportion of disease at time r
x_0 : the proportion of disease at the start of the observation (t = 0)
t : time
r : infection rate of the disease

Analysis the value of area under disease progress curve (AUDPC) and protection index

The Area under Disease Progress Curve (AUDPC) value was obtained based on the intensity of disease infection in a certain observation period. The AUDPC value can describe the level of disease development at a certain time. The AUDPC value was calculated using the following equation 6 (Mehmood and Khan 2016):

$$AUDPC = \sum_{i=1}^{n-1}\left(\frac{X_i - X_{i+1}}{2}\right)(t_{i+1} - t_i) \quad \text{..... (6)}$$

Where:
n : the number of observations
x : the intensity of stalk rot disease, and
(ti+1-ti) : the time interval between observations.

Meanwhile, the protection index was calculated based on the AUDPC value using formula 7 (Caulier et al. 2018)

$$\text{Protection index (\%)} = \left(1 + \frac{\text{AUDPC perlakuan}}{\text{AUDPC kontrol}}\right) \text{x } 100 \quad (7)$$

Lignin and phenol content test of stalk

The lignin content test for the test genotype was carried out using the Klason lignin isolation (Vazquez-Olivo et al. 2019) with modification. 20 cm long stalk samples from each test genotype were taken when the plants were 60 DAP and dried at 70°C for 72 hours. Then, sample was mashed and the materials moisture content was measured. Furthermore, 0.3 g samples were taken and transferred into a test tube, then added 4.5 mL of H_2SO_4 72%. The test tube was shaken at 200 rpm for 2.5 hours. Then, the sample was transferred to a 250 mL Erlenmeyer glass and 171 mL of distilled water was added and covered with aluminum foil. The sample was then autoclaved at 121°C for 15 minutes. After that the sample was filtered using a sterilized filtrate glass. The filtrate glass was washed with 20 mL of water and then acetone. Furthermore, oven at 105°C for 24 hours. After that, the samples were cooled in a desiccator.

The lignin content of corn stalks was calculated based on the following formula (Hartati et al. 2011):

$$\text{Lignin Content} = \frac{C-A}{(100\%-MC) \times B} \times 100 \quad \text{.....(8)}$$

Where:
A : initial glass weight (before filtering)
B : extractive free sample weight
C : weight of glass of filtrate after being used for filtering
MC : moisture content of the sample

The phenolic content of stalks was carried out using the Folin-Ciocalteau method based on Slinkard and Singelton (1977) with modifications. A total of 1 gram of corn stalk sample was crushed until smooth and 2 mL of 96% ethanol was added. A 1 mL of sample was taken to the test tube, after that 1 mL of aquadest and 0.2 mL of folin's reagent (1:10) were added to the solution, vortexed and allowed to stand for 5 minutes. Next, 2 mL of Na_2CO_3 was added, vortexed to make it homogeneous, then allowed to stand for 60 minutes in a dark room. After standing, the absorbance value of solution was measured using UV-Vis spectrophotometry with a wavelength of 765 nm. The standard used was gallic acid and expressed in milligrams of gallic acid equivalent/gram sample (mg GAE/g).

RESULTS AND DISCUSSION

Incidence and severity of corn stalk rot in two season

Symptoms of stalk rot disease in the rainy season begin to be found a week after inoculation. Initial symptoms were included overall leaf wilting, brown discoloration, and soft rot around the inoculated stem. After one or two days of the appearance of these initial symptoms, the plant usually die drooping on the ground (Figure 1). The disease incidence was low at the first observation ranging from 0.71-17.28%. Several genotypes, including MTD1-1, MTD1-5, MTD2-1, MTD2-4, MTD4- 2, MTD4-4, MTD5-1, MTD5-2 and MGOLD were not found infected with stalk rot disease by first week after inoculation. MTD1-1, MTD2-4, MTD4-2 and MTD5-2 genotypes did not become infected with the disease by the second week after inoculation (Table 4). These four genotypes consistently showed the lowest infection until the last observation (6 MSI) with disease incidences of 8.61%, 8.14%, 11.15%, and 15.87% sequentially. Meanwhile, the highest (61.92%) infection was recorded in the MTD5-3 line at 61.92%, which was higher than the two comparison lines. Overall, the results of the evaluation of resistance of 15 hybrid corn lines to stalk rot disease revealed that 5 lines showed resistance, 5 lines reacted with moderate resistance, 3 lines were moderately susceptible, and 2 lines reacted with susceptibility to infection with the disease.

The same genotypes were evaluated during the dry season (June-September 2022). The incidence of stalk rot disease at the beginning of observation was generally higher than at the beginning of observation during the rainy season. Stalk rot infection in the first week after inoculation in the dry season reached 41.33%, and all test lines showed disease infection (Table 5). Disease infection continued to increase until at the time of the last observation (6 MSI), there was only 1 test line with mildly resistant reaction, 14 other lines reacted slightly susceptible to susceptible, and reference line Mal03 reacted to susceptibility to stalk rot disease.

The severity of corn stalk rot disease observed when plants were at 90 DAP in both rainy season and dry season test showed that disease severity was in line with the magnitude of the disease incidence observed previously. The disease severity during dry season was generally higher than in the rainy season, except in the MTD2-1 line (Figure 2). At the time of observation of disease severity, several stalks were found infected with *D. zeae* around the inoculation section, but the overall appearance of plant did not show symptoms of stalk rot. This may be because *D. zeae* did not develop properly in these lines, leading to lower bacterial population.

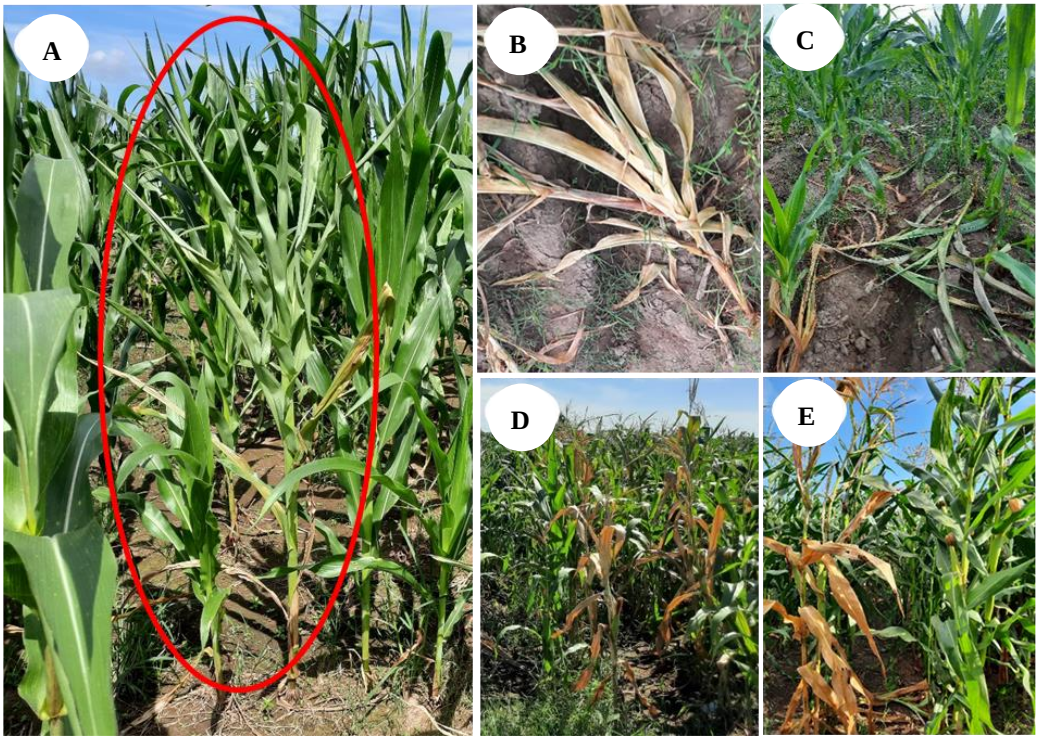

Figure 1. Symptoms of corn stalk rot in field: A. Leaves showing wilting after 1-2 days of inoculation; B-C. Infected plants die and collapsed; D-E. The performance of plants infected with stalk rot among healthy plants in the generative phase of plants.

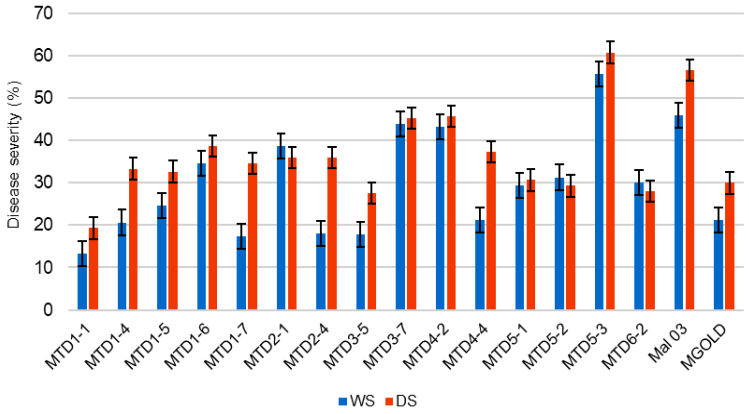

Figure 2. Stalk rot disease severity in 17 genotypes of hybrid corn during WS and DS in Gowa District, South Sulawesi, Indonesia

Table 4. Disease incidence of corn stalk rot in several genotypes of high-yielding potential hybrid maize in Gowa District, South Sulawesi, Indonesia during rainy Season (February-May 2022)

Genotypes	Disease incidence (%)						Resistance criteria
	1 WAI	2 WAI	3 WAI	4 WAI	5 WAI	6 WAI	
MTD1-1	0	0	4.26	4.26	4.26	8.61	R
MTD1-4	1.63	2.53	4.62	4.62	4.62	6.8	R
MTD1-5	0	3.39	6.78	11.18	11.18	21.36	MR
MTD1-6	0.98	2.76	2.76	2.76	10.99	21.11	MR
MTD1-7	1.11	1.11	1.11	2.63	2.63	6.52	R
MTD2-1	0	1.01	1.01	7.15	15.85	26.3	MS
MTD2-4	0	0	0.9	5.26	8.14	8.14	R
MTD3-5	2.47	6.39	10.59	20.15	24.7	41.15	MS
MTD3-7	17.3	17.3	28.4	39.51	44.44	54.32	S
MTD4-2	0	0	2.08	5.12	9.13	11.15	MR
MTD4-4	0	1.11	2.19	3.47	4.54	5.48	R
MTD5-1	0	1.28	4.08	11.76	11.76	13.04	MR
MTD5-2	0	0	1.45	5.63	9.64	15.87	MR
MTD5-3	9.21	11.68	16.53	34.04	37.06	61.92	S
MTD6-2	2.54	2.54	18.05	21.86	25.31	28.17	MS
Mal 03	0.71	2.56	9.69	24.87	37.06	41.62	MS
MGOLD	0	1.28	4.01	8.19	8.19	18.62	MR

Note: WAI: Week after inoculation; R: resistant; MR: moderately resistant; MS: moderately susceptible; S: Susceptible

Table 5. Disease incidence of corn stalk rot in several genotypes of high-yielding potential hybrid maize in Gowa District, South Sulawesi in dry Season (June-September 2022)

Genotypes	Disease incidence (%)						Resistance criteria
	1 WAI	2 WAI	3 WAI	4 WAI	5 WAI	6 WAI	
MTD1-1	11.44	17.14	20.6	22.63	24.01	24.66	MR
MTD1-4	10.75	22.71	26.59	29.11	30.39	31	MS
MTD1-5	30.29	40.35	45.7	46.51	50.41	51.22	S
MTD1-6	25.93	35.7	36.93	42.08	44.05	44.71	MS
MTD1-7	14.54	19.75	23.03	24.88	26.81	26.81	MS
MTD2-1	24.51	34.21	37.05	41.93	44.01	45.46	MS
MTD2-4	10.2	16.34	20.3	23.63	24.96	25.59	MS
MTD3-5	41.33	53.19	59.18	62.9	66.87	66.87	S
MTD3-7	9.96	19.85	42.35	49.76	55.13	55.82	S
MTD4-2	25.95	31.29	34.06	38.68	38.68	38.68	MS
MTD4-4	16.12	26.31	29.74	31.03	32.45	32.45	MS
MTD5-1	15.96	25.15	31.94	33.61	35.3	35.3	MS
MTD5-2	24.17	38.64	41.57	43.45	43.45	43.45	MS
MTD5-3	35.51	52.74	59.22	62.08	66.43	66.43	S
MTD6-2	30.79	33.75	37.45	41.16	44.17	44.17	MS
Mal 03	23.41	40.78	48.26	53.7	57.16	58.01	S
MGOLD	19.78	30.28	34.84	38.06	38.06	38.06	MS

Note: WAI: Week after inoculation; MR: moderately resistant; MS: moderately susceptible; S: Susceptible

Disease development methods

The results showed that incidence of corn stalk rot increased with time and seasonal differences and corn genotype determine the disease development model. The development of stalk rot disease in the rainy season followed the monomolecular, Gompertz and logistic models. Genotypes MTD1-1, MTD1-4, MTD2-1, MTD2-4, MTD4-1, MTD4-4, MTD5-1, MTD5-2, MTD6-2 and MGold followed the monomolecular model. Two genotypes of maize, namely MTD1-6 and MTD1-7 followed the logistic model, and the other four genotypes of maize, such as Mal 03, MTD5-3, MTD3-5 and MTD3-7 followed the Gompertz model of disease development. Whereas, disease development analysis of the stalk rot during dry season in all the test genotypes exhibited the monomolecular disease development model (Table 6).

Based on the results of model accuracy test, it was found that differences in corn genotype did not affect the disease development model during the dry season, but it was found different in rainy season. Differences in corn genotypes influenced the shape of disease development model. Corn genotypes that showed resistant to mild resistant to stalk rot had monomolecular and logistic disease development models. However, maize genotype that reacted slightly susceptible to stalk rot disease had a Gompertz model of disease development, except for strains MTD2-1 and lines MTD6-2. The two lines reacted moderately susceptible, but they both had disease incidence values close to 25% of the maximum limit of moderately resistant criteria.

AUDPC value and protection index

The development of disease incidence values was used as the basis for determining the resistance level of each tested line based on the AUDPC formula. The results of analysis showed that there were differences in the AUDPC and protection index values for 17 maize genotypes. Three maize genotypes showed the lowest AUDPC values both in the rainy and dry season . MTD1-1, MTD1-7 and MTD2-4 with AUDPC values during the rainy season were 757.07, 856.97, 757.63, respectively (Figure 3), while three AUDPC values during the dry season were 119.58, 82.93, and 128.53 (Figure 4). The three maize genotypes consistently showed a protection index value of ≥ 50% in two growing seasons. During the rainy season, protection index values of three genotypes were 57.14%, 51.48%, and 57.10%, and during the dry season protection index values were 82.15%, 87.62%, and 80.81%. Whereas, AUDPC value was significantly higher for the Mal 03 comparator genotype, with a protection index value of 0 in both rainy and dry seasons. This value showed that the Mal 03 genotype was quite susceptible to *D. zeae* infection (Table 7).

Effect of stalk lignin and phenol content on the incidence of stalk rot disease

A correlation test of lignin and phenol content on the incidence of stalk rot showed that both biochemical showed a negative correlation with disease incidence. The correlation values were -0.60877 and -0.06047 (Figures 5 and 6). Lignin content showed a strong correlation with disease incidence. This means that the higher the lignin content of corn stalks, the lower the incidence of stalk rot disease, in contrast to the phenol content, which was very weakly correlated.

Table 6. The accuracy of disease development model of corn stalk rot in two growing seasons (WS and DS) in Gowa District, South Sulawesi, Indonesia

Genotypes	Wet season			Dry season		
	Disease development models	Regression	R^2	Disease development models	Regression	R^2
MTD1-1	Monomolecular	Y = 0.00237x - 0.0213	0.85	Monomolecular	Y=0.00447x + 0.1163	0.90
MTD1-4	Monomolecular	Y = 0.0014x - 0.0088	0.88	Monomolecular	Y = 0.00668x + 0.1294	0.81
MTD1-5	Monomolecular	Y = 0.0061x - 0.0530	0.91	Monomolecular	Y = 0.00961x + 0.3534	0.90
MTD1-6	Logistic	Y = 0.00853x - 5.2076	0.88	Monomolecular	Y = 0.00802x + 0.2905	0.90
MTD1-7	Logistic	Y = 0.00516x - 5.1557	0.83	Monomolecular	Y = 0.00439x + 0.1507	0.90
MTD2-1	Monomolecular	Y = 0.00848x - 0.1124	0.83	Monomolecular	Y = 0.00894x + 0.2631	0.93
MTD2-4	Monomolecular	Y = 0.00295x - 0.03360	0.89	Monomolecular	Y = 0.00534x + 0.0967	0.91
MTD3-5	Gompertz	Y = 0.03879x - 1.5867	0.98	Monomolecular	Y =0.01629x + 0.4991	0.92
MTD3-7	Gompertz	Y = 0.03226x - 0.8868	0.97	Monomolecular	Y = 0.0222x - 0.0132	0.92
MTD4-2	Monomolecular	Y = 0.00371x - 0.0430	0.94	Monomolecular	Y = 0.00554x + 0.2908	0.87
MTD4-4	Monomolecular	Y = 0.00164x - 0.0150	1.00	Monomolecular	Y = 0.00556x + 0.1955	0.77
MTD5-1	Monomolecular	Y = 0.00457x - 0.0038	0.90	Monomolecular	Y = 0.00722x + 0.1779	0.83
MTD5-2	Monomolecular	Y = 0.00960x - 0.1334	0.66	Monomolecular	Y = 0.00712x + 0.3277	0.66
MTD5-3	Gompertz	Y = 0.79190x - 3.0415	0.96	Monomolecular	Y = 0.01781x + 0.4366	0.88
MTD6-2	Monomolecular	Y = 0.00968x - 0.0505	0.92	Monomolecular	Y = 0.00673x + 0.3259	0.96
Mal 03	Gompertz	Y = 0.05343x - 1.9652	0.98	Monomolecular	Y = 0.01668x + 0.2471	0.91
MGOLD	Monomolecular	Y = 0.00406x - 0.0176	0.63	Monomolecular	Y = 0.00693x + 0.2379	0.78

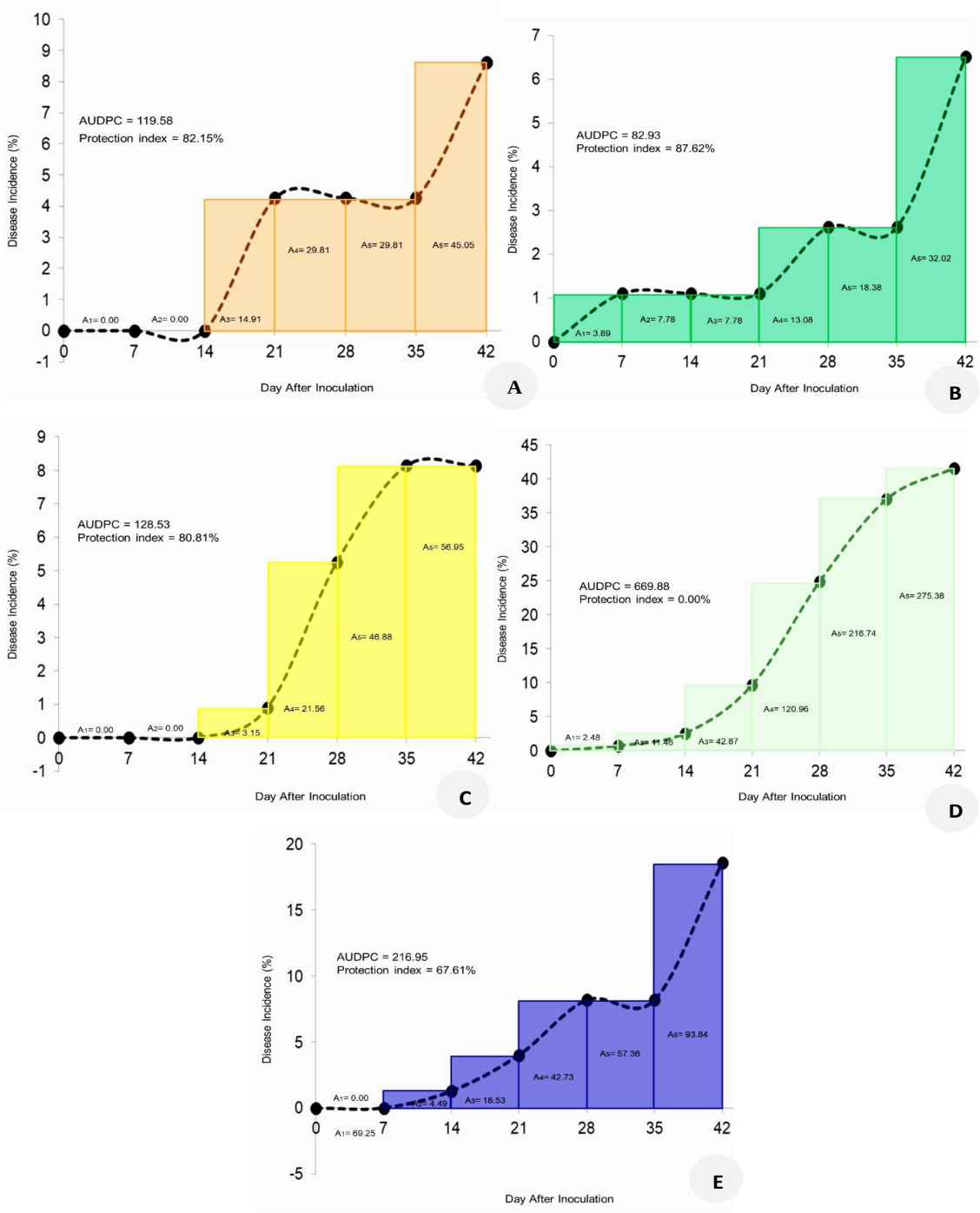

Figure 3. Effect of high-yielding potential hybrid maize lines on AUDPC and protection index in South Sulawesi, Indonesia during rainy season: A. MTD1-1 genotype; B. MTD1-7 genotype; C. MTD2-4 genotype; D. Mal 03 genotype; E. MGOLD genotype

Discussion

Stalk rot disease on corn is one of those diseases that need to be aware of because it causes high yield loss. Infected plants wither and eventually die. Infected plants become wilted similar to the symptoms of damage due to high water stress (Ansermet et al. 2016). Results showed that resistance reaction of the test lines to stalk rot disease varied both in the WS and DS. These differences are due to plant genetic variations that can support or suppress disease development. Gudero et al. (2018) stated that genetic variation in tomatoes and environmental factors could cause differences in the severity of tomato late blight disease. The incidence of corn stalk rot in the dry season was generally found to be higher than the disease incidence during the rainy season. This is different from the statement by Kumar et al. (2017) that corn stalk rot disease was found to infect plants during the rainy season. The high incidence of disease in the dry season in this study was due to the use

of the same land for planting in both seasons. The implementation time was very short, dry season planting was done ± 15 days after the rainy season crops were harvested. *D. zeae* is a soil-borne bacterium and can survive as an epiphyte or saprophyte in the soil and groundwater until a suitable host is found (Reverchon and Nasser 2013; Kumar et al. 2017). Furthermore, Adesh et al. (2017) found that *D. zeae* can survive up to 270 days in field soil and sterilized soil when mixed with plant residues infected with stalk rot. The bacterial inoculum from infected plants in the rainy season persists in the soil and begins to infect dry-season crops. Bacterial accumulation through artificial inoculation and the presence of bacteria in the field were the two important factors for the high incidence of disease during the dry season. Disease development after inoculation is influenced by several factors, including plant age, inoculum concentration, host resistance, bacterial strain, and environmental factors (Adorada et al. 2013).

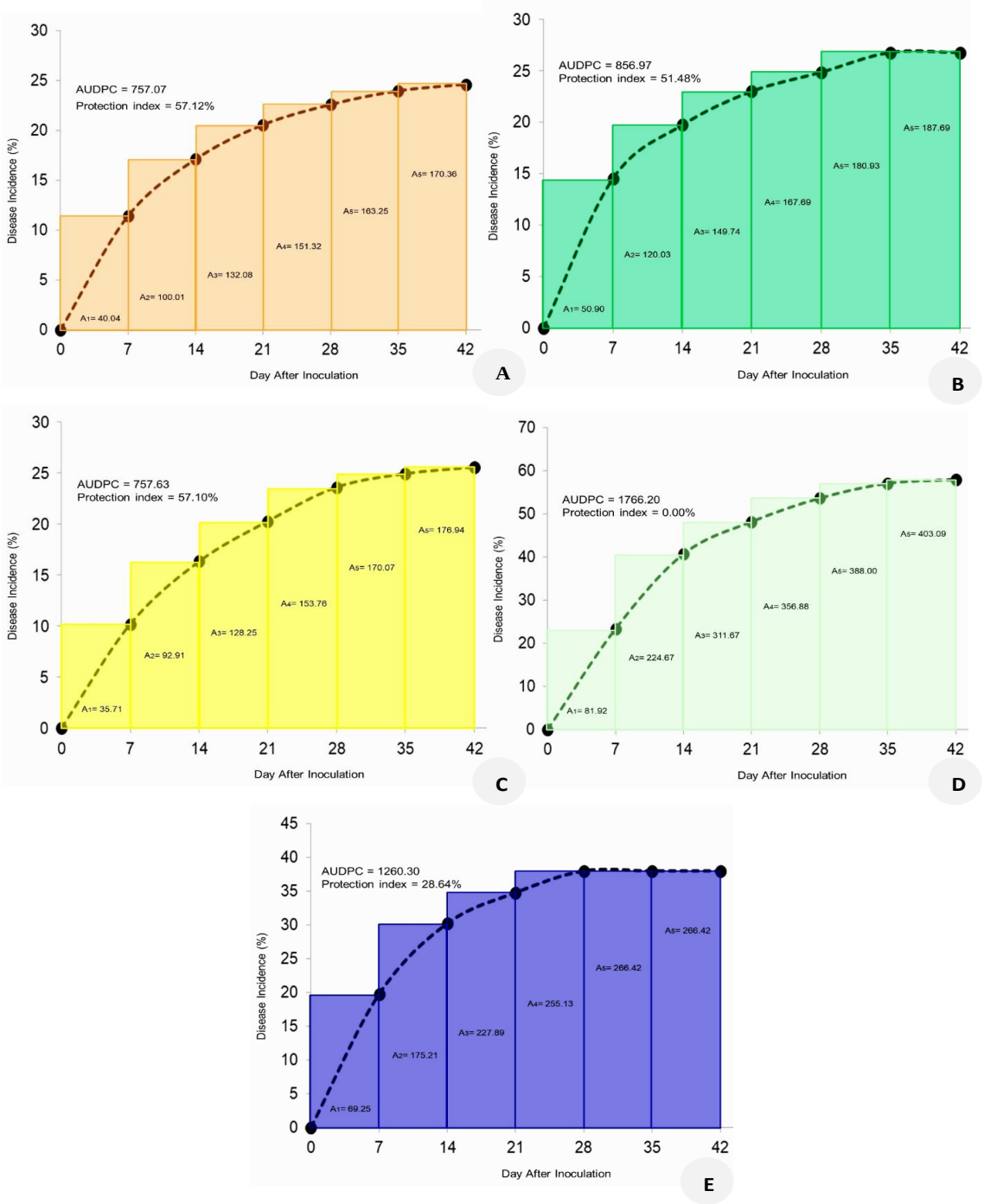

Figure 4. Effect of high-yielding potential hybrid maize lines on AUDPC and protection index in South Sulawesi, Indonesia during dry season; (a) MTD1-1 genotype; (b) MTD1-7 genotype; (c) MTD2-4 genotype; (d) Mal 03 genotype; (e) MGOLD genotype

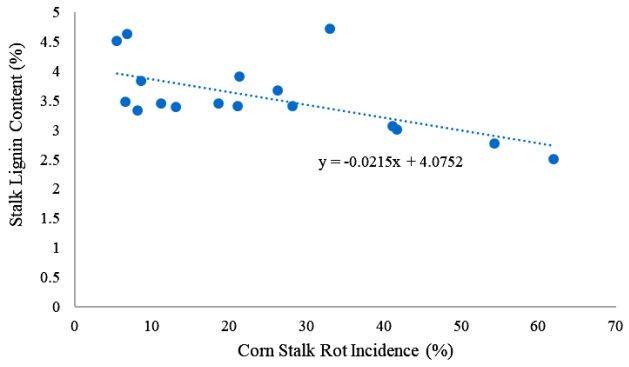

Figure 5. Correlation of corn stalk rot incidence with stalk lignin content of 17 hybrid maize lines

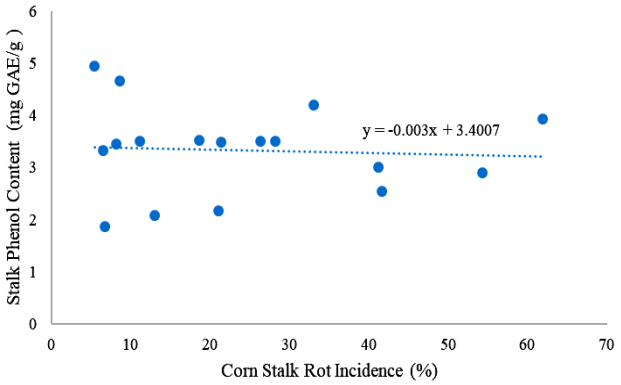

Figure 6. Correlation of corn stalk rot disease incidence with stalk phenol content of 17 hybrid maize lines

Table 7. Effect of hybrid maize genotype on AUDPC value and maize stalk rot disease protection index in WS and DS

Genotypes	Wet Season		Dry Season	
	AUDPC	Protection Index (%)	AUDPC	Protection Index (%)
MTD1-1	119.58	82.15	757.07	57.14
MTD1-4	149.95	77.62	945.29	46.48
MTD1-5	302.53	54.84	1672.09	5.33
MTD1-6	215.65	67.81	1449.34	17.94
MTD1-7	82.93	87.62	856.97	51.48
MTD2-1	267.19	60.11	1431.09	18.97
MTD2-4	128.53	80.81	757.63	57.10
MTD3-5	594.08	11.32	2218.30	-25.60
MTD3-7	1218.52	-81.90	1434.67	18.77
MTD4-2	153.31	77.11	1316.09	25.49
MTD4-4	98.36	85.32	1063.05	39.81
MTD5-1	247.84	63.00	1117.31	36.74
MTD5-2	232.67	65.27	1491.03	15.58
MTD5-3	981.05	-46.45	2164.41	-22.54
MTD6-2	590.64	11.83	1465.79	17.01
Mal 03	669.88	0.00	1766.22	0.00
MGOLD	216.95	67.61	1260.32	28.64

The results of model analysis of development of corn stalk rot disease showed that disease development during dry season followed the monomolecular model, in contrast to the rainy season, where several strains followed the logistic and Gomperzt models. These different models reflect differences in the speed of disease progression and the cycle of pathogens. This indicates that although the pathogen causing corn rot is soil-borne, environmental factors such as the genotype of maize affect the infection cycle. Appropriate environmental support can cause repeated cycles of infection or inoculums from diseased plants can infect surrounding plants, causing soil-borne pathogens which that generally follow the monomolecular disease development model and can be found in other models in the field (Bande et al. 2015). Furthermore, disease development model can determine the value of infection rate. The infection rate of stalk rot disease in present study showed that the lower the infection rate, the more resistant the strain, but conversely, the higher the infection rate, the more susceptible the strain. Test genotypes MTD3-5, MTD3-7, MTD5-3 and control genotype Mal 03 had the highest corn stalk rot infection rate both in rainy and dry seasons. The infection rates of MTD3-5, MTD3-7, MTD5-3 and Mal 03 maize genotypes during rainy season were 0.03879, 0.03226, 0.79190, and 0.05343, respectively. The disease infection rates that occurred in the four genotypes of maize during dry season were 0.01629, 0.02220, 0.01781, and 0.01668. The infection rate value was linear with the resistance level of the test genotypes MTD3-5, MTD3-7, MTD5-3, and the control genotype Mal 03 to stalk rot which reacted from susceptibility to very susceptibility. In addition, strains using the monomolecular disease development method showed an overall lower the infection rate during the rainy season compared to the disease infection rate during the dry season. This shows that the higher the incidence of disease at the start of the observation, the higher the infection rate. Disease infection rate can be suppressed through several methods including reducing inoculum production, infection rate, or development of pathogens by determining unfavorable growing seasons for pathogens, reducing inoculum from external sources during epidemics, and assembling resistant varieties (Arya 2018).

Knowledge of plant pathogen resistance and immunity is very useful in determining control measures (Andersen et al. 2018). Khandare et al. (2018) reported that necrosis disease in sunflowers begins to develop when the plant is 30 HST and lasts throughout the season, but the disease develops very slowly in the early stages of plant growth and then increases with increasing plant age. Further analysis of AUDPC values and protection index values showed that 3 hybrid maize lines (lines MTD1-1, MTD1-7, and MTD2-4) had narrow disease development areas with consistent protection index values above ≥ 50% in both growing seasons. This illustrates that the development of stalk rot disease was quite slow along these lines.

According to Astiko and Sudantha (2023), the narrower the area for disease development, the more resistant the plant. The difference in AUDPC values for each line was may be due to differences in plant morphology and physiology. The present study analyzed two plant characters and showed that contents of lignin and phenol in the stalks were negatively correlated with corn stalk rot disease. This showed that the higher the lignin and phenol content of stalk, the lower the chance of stalk rot disease infection. However, lignin content of stalk had a stronger effect than the phenol content as indicated by a correlation value of -0.60877. Previous research conducted by Liu and He (2010) showed that lignin content induced by the application of KCl fertilizer could increase the resistance of maize plants to stalk rot disease caused by *Fusarium verticilloides*. Tomato plants resistant to disease caused by bacterium *Ralstonia solanacearum* have a higher lignin content than susceptible cultivars (Mandal et al. 2013). This suggests that lignin biosynthesis contributes extensively to plant resistance to biotic and abiotic stresses, including pathogenic infections. According to Ma et al. (2017) and Miedes et al. (2014), accumulation of lignin can suppress the activity of pathogen infection in host plants and prevent the multiplication and movement of these pathogens.

Based on the results of this research, it was found that three maize lines (lines MTD1-1, MTD1-7, and MTD2-4) could potentially be used as material for the production of new corn varieties resistant to stalk rot. The content of lignin and phenol in stalks were negatively correlated with disease incidence, but lignin content had a strong correlation. Thus, these characteristics can be considered in selecting and developing plants resistant to bacterial stalk rot. Genetic improvement of plants through breeding for disease resistance is a sustainable strategy and can reduce farmers' losses.

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CURRICULUM VITAE

A. IDENTITAS DIRI

Nama Lengkap : **Dr. Suriani, S.P., M.P.**

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Pangkat/Golongan : **Penata / IIIc**

Jenis Kelamin : **Perempuan**

Agama : **Islam**

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B. RIWAYAT PENDIDIKAN

No	Tingkat	Nama Pendidikan	Jurusan	Tahun
1.	Dasar	SD 185 Cilellang	-	1991-1997
2.	Menengah Pertama	SMP 3 Panincong	-	1997-2000
3.	Menengah Atas	SMA 1 Donri-Donri	IPA	2000-2003
4.	Strata 1 (S1)	Fakultas Pertanian Unhas	Ilmu Hama & Peny. Tanaman	2003-2007
5.	Strata 2 (S2)	Sekolah Pascasarjana Unhas	Sistem-sistem Pertanian	2007-2009
6.	Strata 3 (S3)	Sekolah Pascasarjana Unhas	Ilmu Pertanian	2020-2023

C. PENGALAMAN PENELITIAN 5 TAHUN TERAKHIR

No	Judul Penelitian	Tahun
1	Pengembangan Produk Bio-Inhibitor Berbahan Aktif *Trichoderma asperellum* dan *Penicillium Raperi* untuk Pengendalian Patogen Tular Tanah pada Tanaman Jagung (Sumber Dana: BRIN).	2023
2	Uji Adaptasi dan Ketahanan Penyakit Utama Calon Varietas Jagung Hibrida Kerjasama Fakultas Pertanian UNHAS dengan CV. Trubus Gumelar (Sumber Dana: CV. Trubus Gumelar)	2023
3	Uji Adaptasi dan Ketahanan Penyakit Utama Calon Varietas Jagung Hibrida Kerjasama Fakultas Pertanian UNHAS dengan PT. MAXXI (Sumber Dana: PT. MAXXI)	2023
4	Uji ketahanan Penyakit bulai (*Peronosclerospora phillippinensis*) calon varietas jagung hibrida, Kerjasama Fakultas Pertanian UNHAS dengan PT. Syngenta Indonesia (Sumber Dana: PT. Syngenta Indonesia)	2023

5	Perakitan varietas jagung hibrida potensi hasil tinggi (13,75 ton/ha) tahan hama dan penyakit utama (Sumber Dana: LPDP)	2023
6	Uji Adaptasi dan Ketahanan Penyakit Utama Calon Varietas Jagung Hibrida Kerjasama Fakultas Pertanian UNHAS dengan PT. Protani Gemilang Indonesia (Sumber Dana: PT. Protani Gemilang Indonesia)	2023
7	Uji Adaptasi dan Ketahanan Penyakit Utama Calon Varietas Jagung Hibrida Silang Tunggal Kerjasama Fakultas Pertanian UNHAS dengan PT. Jafran (Sumber Dana: PT. Protani Gemilang Indonesia)	2023
8	Karakterisasi bakteri penyebab penyakit busuk batang jagung di Indonesia dan pembentukan galur jagung potensi hasil tinggi tahan penyakit busuk batang	2022
9	Pengembangan Teknologi Nano Bio-Inhibitor sebagai Pengendali Patogen Tular Tanah Utama untuk Mendukung Peningkatan Produksi Jagung Nasional	2022
10	Aplikasi Konsorsium Mikroba Indigenous sebagai Agens Hayati Penyakit Busuk Batang Jagung	2020
11	Pemanfaatan Bahan Elisitor Sebagai Penginduksi Ketahanan Tanaman Jagung terhadap Patogen Karat Daun (*Puccinia* spp)	2020
12	Evaluasi ketahanan calon varietas unggul jagung hibrida terhadap penyakit utama kerjasama PT. Dupont	2020
13	Pengendalian terpadu dengan kombinasi fungisida sintetik dan pupuk kalsium untuk menekan penyakit bulai (*Peronosclerospora philipinensis*)	2020
14	Evaluasi ketahanan genotipe jagung hibrida terhadap penyakit utama	2019
15	Kombinasi Biopestisida Formulasi *Bacillus Subtilis* TM3 Dan Pestisida Nabati Dalam Mengendalikan Penyakit Busuk Batang Fusarium Pada Tanaman Jagung	2019
16	Evaluasi ketahanan calon varietas unggul jagung hibrida terhadap penyakit utama kerjasama PT. Syngenta	2019
17	Evaluasi ketahanan calon varietas unggul jagung hibrida terhadap penyakit utama kerjasama PT. MSN	2019
18	Evaluasi Penyakit Utama yaitu Karat (*Puccinia purpurea*), Bercak Daun Cercospora (*Cercospora sorghi*) Antraknosa (*Colletotrichum graminicola*) dan Busuk Batang *pada Beberapa Calon varietas baru* Sorgum Institute Pertanian Bogor (IPB)	2019
19	Evaluasi Penyakit Utama yaitu Karat (*Puccinia purpurea*), Bercak Daun Cercospora (*Cercospora sorghi*) Antraknosa (*Colletotrichum graminicola*) dan Busuk Batang *pada Beberapa Calon varietas baru* Sorgum PAIR-BATAN	2019
21	Efektivitas formulasi *Bacillus subtilis* untuk mengendalikan penyakit busuk batang fusarium (*Fusarium verticilloides*) pada pertanaman jagung	2018
22	Evaluasi ketahanan galur, populasi Varietas Turunan Esensial Lamuru, Sukmaraga, dan Bisma Produktivitas Tinggi terhadap penyakit bulai	2018
23	Evaluasi ketahanan genotipe jagung hibrida ungu terhadap penyakit bulai	2018
24	Evaluasi Penyakit Utama yaitu Karat (*Puccinia purpurea*), Bercak Daun Cercospora (*Cercospora sorghi*) Antraknosa (*Colletotrichum graminicola*) dan busuk batang *pada* Galur Mutan Sorgum	2018

D. HKI/ PATEN

No	Judul Paten	Tahun
1.	Tribas, Formula Biopestisida Tepung Berbahan Aktif *Bacillus subtilis*	2016
2.	SK pelepasan VUB Jagung JH47	2017
3.	SK pelepasan VUB Jagung JH35	2017
4.	SK Pelepasan VUB Sorgum Bioguma 2 Agritan	2019
5.	SK Pelepasan VUB Sorgum Bioguma 1 Agritan	2019
6.	SK Pelepasan VUB Sorgum Bioguma 3 Agritan	2019
7.	SK pelepasan VUB Jagung PUSAKA 1	2021

E. KARYA TULIS ILMIAH

1. Publikasi Jurnal Internasional Bereputasi

No	Judul Karya Ilmiah
1.	*Molecular characterization of indigenous microbes and its potential as a biological control agent of Fusarium stem rot disease (Fusarium verticillioides) on maize.* Heliyon, 2022, 8(12), e11960. terindeks scopus QI. https://doi.org/10.1016/j.heliyon.2022.e11960
2.	*Indigenous fungi from corn as a potential plant growth promoter and its role in Fusarium verticillioides suppression on corn.* Heliyon 7 (2021) e07926, terindeks scopus QI. https://doi.org/10.1016/j.heliyon.2021.e07926
3.	*Control of Fusarium verticillioides on corn with a combination of Bacillus subtilis TM3 formulation and botanical pesticides*. Saudi Journal of Biological Sciences, Volume 28, Issue 12, December 2021, Pages 7000-7005, terindeks scopus QI. https://doi.org/10.1016/j.sjbs.2021.07.083
4.	*Morpho-physiological and molecular characteristics of bacteria causing stalk rot disease on corn in Gorontalo, Indonesia.* *Biodiversitas Vol. 24 No. 3 (2023. Terindeks scopus Q3. https://doi.org/10.13057/biodiv/d240349*
5.	*New corn resistant lines to stalk rot disease (Dickeya zeae) in Indonesia.* *Biodiversitas Vol. 24 No. 6 (2023). Terindeks scopus Q3. https://doi.org/10.13057/biodiv/d240612*

2. Prosiding Internasional

No	Judul Karya Ilmiah
1.	*The performance of sorghum mutant lines resulting from gamma-ray mutation on main diseases* International Conference of Post Graduate Program, University of Papua (ICOPOD 2022) 24/11/2022 - 24/11/2022 Raja Ampat, Indonesia. https://doi.org/10.1088/1755-1315/1192/1/012003.
2.	*The presence of bacterial stalk rot disease on corn in Indonesia: A review.* IOP Conference Series: Earth and Environmental Science, Volume 911012058, 2nd International Conference on Sustainable Cereals and Crops Production System in the Tropics 23-25 September 2021. https://iopscience.iop.org/article/10.1088/1755-1315/911/1/012058/meta
3.	*Genotype resistance of hybrid corn varieties candidate against major corn diseases.* IOP Conference Series: Earth and Environmental Science, Volume 911012058, 2nd International Conference on Sustainable Cereals and Crops Production System in the Tropics 23-25 September 2021. https://iopscience.iop.org/article/10.1088/1755-1315/911/1/012054/meta
4.	*Variations of cob rot infection caused by Fusarium verticillioides in the Filial 1(F1) hybrid maize line*. IOP Conference Series: Earth and Environmental Science, Volume 911012058, 2nd International Conference on Sustainable Cereals and Crops Production System in the Tropics 23-25 September 2021. https://iopscience.iop.org/article/10.1088/1755-1315/911/1/012057/meta
5.	*Incorporating adoption of agricultural bio-innovation in a famers' participatory parental seed production*. E3S Web of Conferences 306, 01028 (2021), 1st ICADAI 2021. https://doi.org/10.1051/e3sconf/202130601028
6.	*Components of environment affecting the reproduction of powder beetle Sitophilus zeamais (motsch.).* E3S Web of Conferences, 2022, 361, 04025

7.	*An in-depth study on Sitophilus zeamais Motsch (Coleoptera: Curculionidae) pests on corn plants* IOP Conference Series: Earth and Environmental Sciencethis link is disabled, 2022, 1107(1), 012060
8.	*Effectiveness of Bacillus subtilis TM4 biopesticide formulation as biocontrol agent against maydis leaf blight disease on corn.* IOP Conf. Series: Earth and Environmental Science 484 (2020) 012096
9.	*Utilization of antagonistic bacteria Bacillus subtilis to control Fusarium verticilloides on corn.* IOP Conf. Series: Earth and Environmental Science 484 (2020) 012096
10.	*The presence of bacterial stalk rot disease on corn in Indonesia: A review.* IOP Conference Series: Earth and Environmental Science, Volume 911012058, 2nd International Conference on Sustainable Cereals and Crops Production System in the Tropics 23-25 September 2021. https://iopscience.iop.org/article/10.1088/1755-1315/911/1/012058/meta

3. Jurnal Nasional

No	Judul Karya Ilmiah
1.	*The Response of Foxtail Millet Candidate Varieties from Nagekeo Regency to Leaf Blight (Bipolaris setariae)* Agrosainstek, 5 (1) 2021: 1-7. https://doi.org/10.33019/agrosainstek.v5i1.207
2.	*Ketahanan Beberapa Genotipe Jagung Hibrida Umur Genjah terhadap Sitophilus zeamais Motschulsky* Jurnal Agronomi Indonesia Volume 47 (1) April 2019: 18-24. https://doi.org/10.24831/jai.v47i1.21170
3.	*Exploration and screening for endophytic microbes of maize plant root against Fusarium verticillioides* Jurnal HPT Tropika Volume 18 (1) Maret 2018. https://doi.org/10.23960/j.hptt.11857-64
4.	*Efikasi formulasi Bacillus subtilis terhadap pengendalian penyakit busuk batang Fusarium pada tanaman jagung* Jurnal Penelitian Pertanian Tanaman Pangan Volume 2 No. 3 Desember 2018:191-197. http://dx.doi.org/10.21082/jpptp.v2n3.2018.p191-197
5.	*Correlation of Stomata Density To Rust Severity On Some Accessions Of Maize Germplasm* Jurnal HPT Tropikas Volume 18 (2) September 2019. https://doi.org/10.23960/j.hptt.21895-104
6.	*Kombinasi Aplikasi Biopestisida dan Pestisida Nabati untuk Mengendalikan Penyakit Hawar Daun Bipolaris maydis pada Jagung* Jurnal Penelitian Pertanian Tanaman Pangan Volume 2 Nomor 1 Tahun 2018. http//dx.doi.org/10.21082/jpptp.v2n1.2018.p43-49
7.	*Prospek Bacillus subtilis sebagai agen pengendali patogen tular tanah pada tanaman jagung.* Jurnal Penelitian dan Pengembangan Pertanian Volume 35 Nomor 1 Tahun 2016. http://dx.doi.org/10.21082/jp3.v35n1.2016.p37-45

4. Prosiding Nasional

No	Judul Karya Ilmiah
1.	*Reaksi Ketahanan Beberapa Genotipe Calon Varietas Jagung Hibrida terhadap Tiga Penyakit Utama Jagung*

	Prosiding Seminar Nasional Fakultas Pertanian UNS, "Membangun Sinergi antar Perguruan Tinggi dan Industri Pertanian dalam Rangka Implementasi Merdeka Belajar Kampus Merdeka". 12 Juni 2021
2.	*Respon Ketahanan Beberapa Calon Varietas Jagung Hibrida Jagung Hibrida terhadap 3 Penyakit Utama Jagung* Prosiding Seminar Nasional Pertanian Peternakan Terpadu Ke-3 "Peningkatan Daya Saing Sumber Daya Lokal di Era Revoluasi Industri 4. 0". Universitas Muhammadiyah Purworejo, 14 Maret 2020
3.	*Respon Ketahanan Beberapa Aksesi Sorgum Manis Hasil Iradiasi Terhadap Penyakit Utama Sorgum* Prosiding Seminar Nasional Virtual "Peningkatan Produkstivitas Pertanian Indonesia Melalui Penerapan Teknologi Pengendalian Hama Penyakit Tumbuhan Sumbangan Hasil Penelitian Perguruan Tinggi dan Lembaga Pengembangan Pertanian. urusan Perlindungan Tanaman Fakultas Pertanian Universitas Bengkulu. 23-25 Juni 2020

6. Buku

Penerbit	Judul Buku	Tahun Terbit
PENERBIT DEEPUBLISH (Grup Penerbitan CV BUDI UTAMA) Anggota IKAPI (076/DIY/2012)	Penyakit Bulai Pada Tanaman Jagung dan Upaya Pengendaliannya	2018

Saya menyatakan bahwa semua keterangan dalam Curriculum Vitae ini adalah benar dan apabila terdapat kesalahan, saya bersedia mempertanggungjawabkannya.

Makassar, 14 Agustus 2023
Yang Bersangkutan

Dr. Suriani, S.P., M.P.