BIODIVERSITAS
Volume 24, Number 6, June 2023
Pages: 3190-3200

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240612

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

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Manuscript received: 31 March 2023. Revision accepted: 12 June 2023.

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.

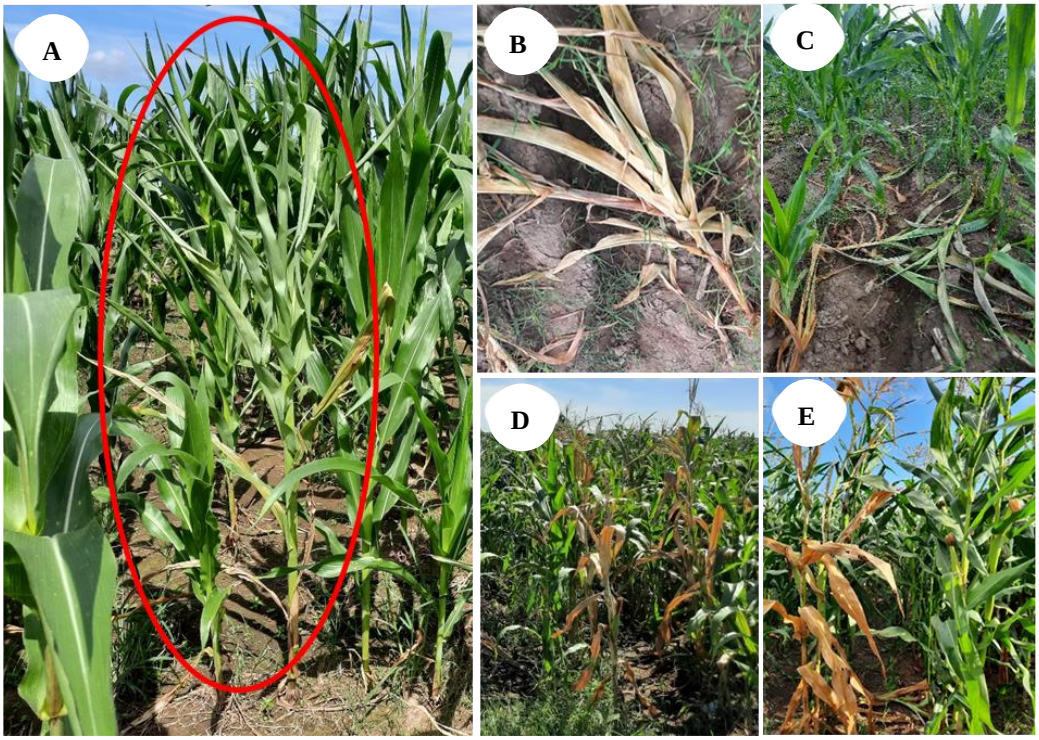

Figure 1. Symptoms of corn stalk rot in field: A. Leaves showing wilting after 1-2 days of inoculation; B-C. Infected plants die and collapsed; D-E. The performance of plants infected with stalk rot among healthy plants in the generative phase of plants.

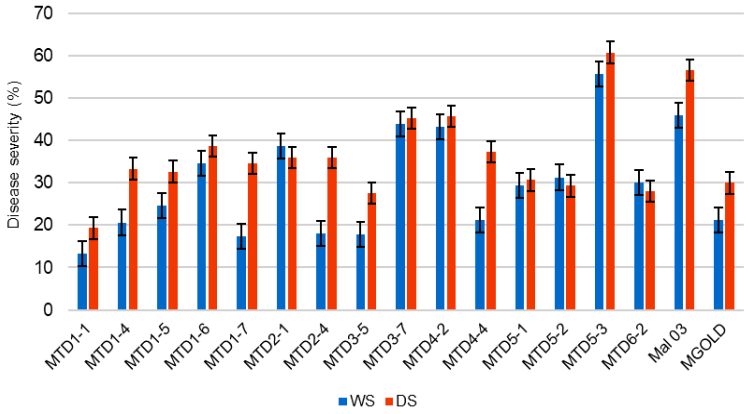

Figure 2. Stalk rot disease severity in 17 genotypes of hybrid corn during WS and DS in Gowa District, South Sulawesi, Indonesia

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

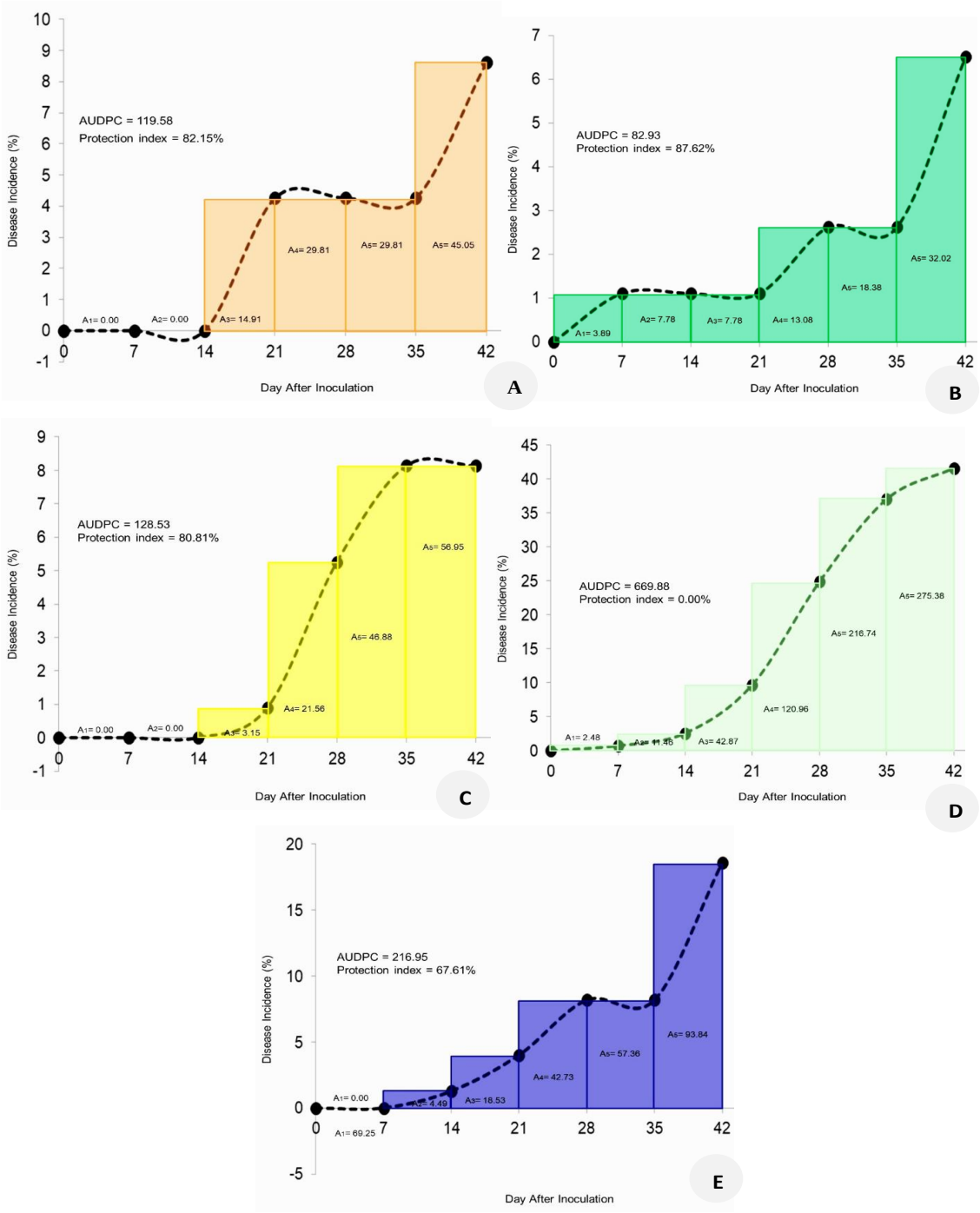

Figure 3. Effect of high-yielding potential hybrid maize lines on AUDPC and protection index in South Sulawesi, Indonesia during rainy season: A. MTD1-1 genotype; B. MTD1-7 genotype; C. MTD2-4 genotype; D. Mal 03 genotype; E. MGOLD genotype

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).

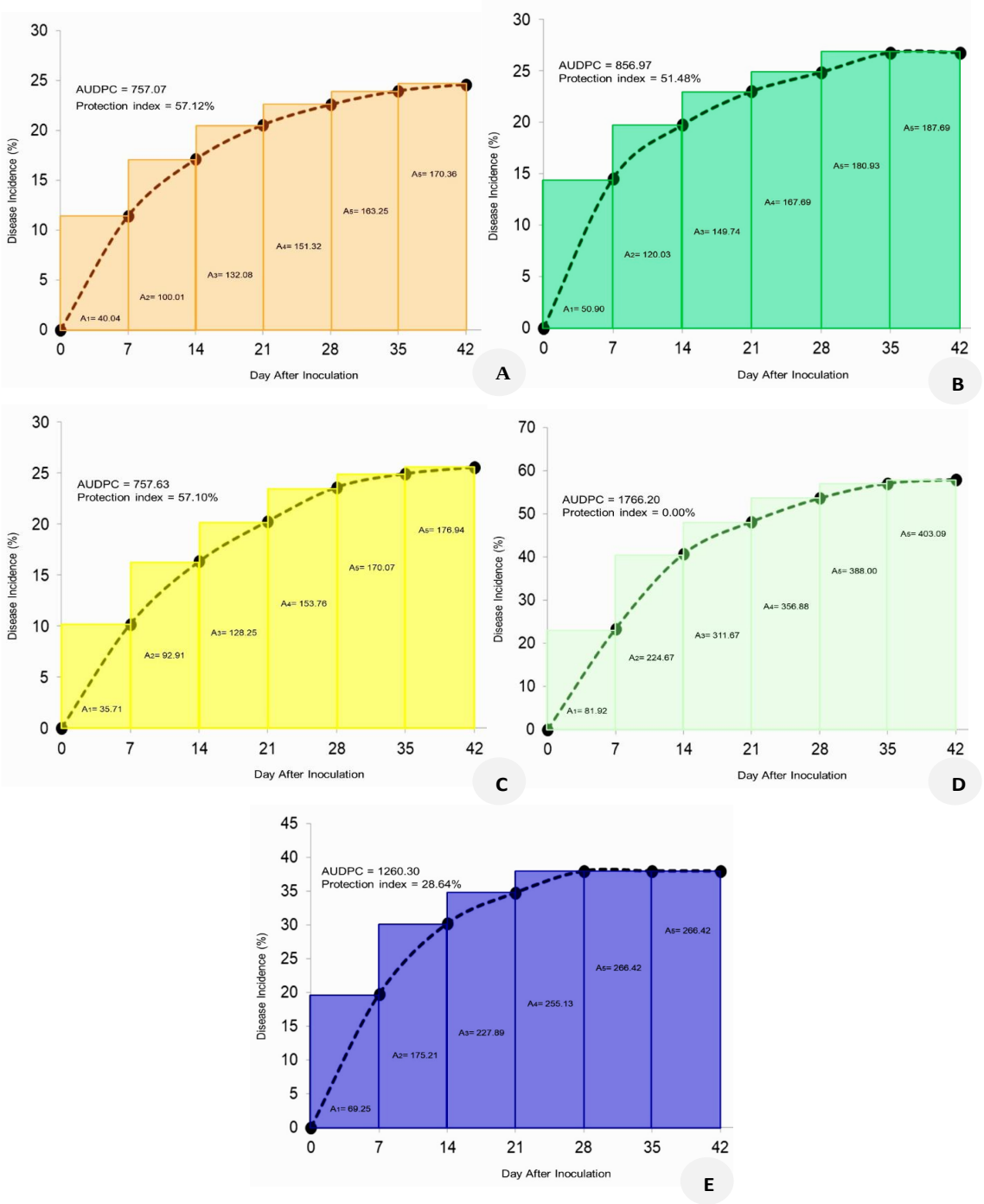

Figure 4. Effect of high-yielding potential hybrid maize lines on AUDPC and protection index in South Sulawesi, Indonesia during dry season; (a) MTD1-1 genotype; (b) MTD1-7 genotype; (c) MTD2-4 genotype; (d) Mal 03 genotype; (e) MGOLD genotype

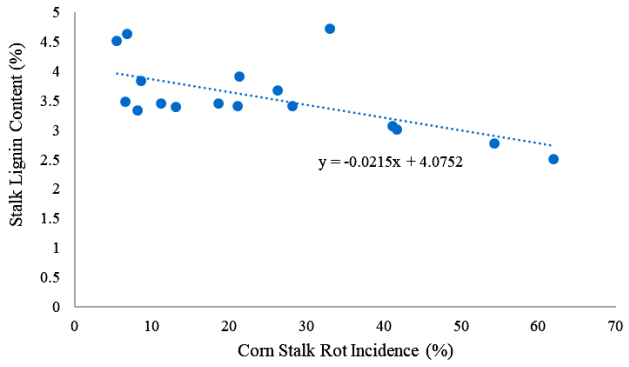

Figure 5. Correlation of corn stalk rot incidence with stalk lignin content of 17 hybrid maize lines

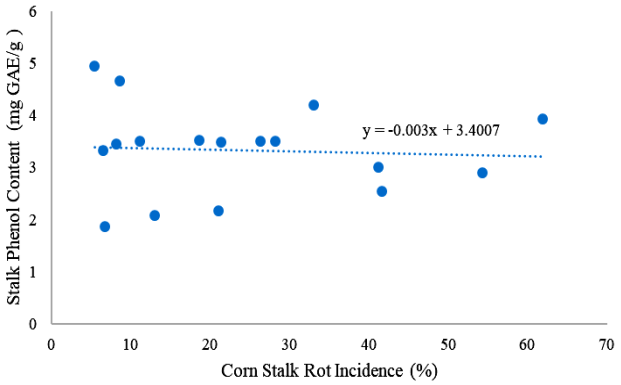

Figure 6. Correlation of corn stalk rot disease incidence with stalk phenol content of 17 hybrid maize lines

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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