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Identification of *Rhizoctonia solani* The Caused of Corn Sheath Blight in Central Java

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ABSTRACT

Corn (*Zea mays* L.) is a food crop in Indonesia, one of the factors of decreased production is plant diseases, one of which is sheath blight caused by *Rhizoctonia solani*. The purpose of this study was to identify corn sheath blight caused by *Rhizoctonia solani* and to assess the pathogenicity and virulence of *Rhizoctonia solani* isolates. The research methods used included the exploration stage of sheath blight disease in several areas in Central Java, macroscopic and microscopic morphological identification, molecular identification using specific primers, and pathogenicity tests. The results of the study showed that, at the exploration stage, the symptoms observed included widespread tissue damage in the form of lesions and the presence of sclerotia. Morphological identification of the mycelial distribution pattern was scarce, the sclerotia distribution pattern was on the peripheral and scattered, the branching angle was 90º, and in the multinucleate category. Molecular identification showed that sheath blight was caused by *Rhizoctonia solani* AG1-IA. Pathogenicity test showed the fastest incubation period in isolates from Boyolali, at 5,80 days after inoculation; incidence reached 100% at week 4; the highest intensity in isolates from Boyolali was 73%. The conclusion is the pathogen causing corn sheath blight in Central Java is *Rhizoctonia solani* AG1-IA, and the pathogenicity test results showed that the Boyolali isolate had the highest intensity and incubation period.

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Introduction:

Corn (*Zea mays* L.) is a commodity in the agricultural sector whose demand continues to increase. Projected corn consumption in Indonesia is projected to increase in 2023, 2024, and 2025, reaching 14.7, 15.2, and 15.5 million tons, respectively (Ministry of Agriculture, 2025). Corn production centers in Java, including the provinces of East Java, Central Java, and West Java, are only capable of producing 4.57 million, 2.29 million, and 639 thousand tons per year, respectively (Ministry of Agriculture, 2024). Meeting this corn demand can be hampered by challenges, including plant diseases during cultivation. Sheath blight, caused by *Rhizoctonia solani*, is a major corn disease (Singh *et al*., 2018). Symptoms of corn plants infected with *R. solani* include widespread tissue damage (necrosis) with irregular edges. Other symptoms during the germination phase include brown corn roots that grow smaller and dry out (Shi *et al*., 2021). Signs of *R. solani* infection include the presence of sclerotia on the affected plant parts. *R. solani* sclerotia are a collection of thickened and filling hyphae (Moni *et al*., 2016).

Sheath blight disease in Indonesia causes significant losses. Therefore, sheath blight caused by *R. solani* must be controlled to avoid crop failure. Sheath blight can cause up to 50% of corn yield losses if left untreated. Losses due to disease in corn fields in the United States and Canada are 10.9%, and losses due to *R. solani* are 4% of the total disease burden in corn crops (Mueller *et al*., 2016). *Rhizoctonia solani* isolates exhibit high disease severity. The *R. solani* isolate from Klaten exhibits the highest virulence, with a disease severity rate reaching 25% (Desvani *et al*., 2018). Effective control of sheath blight requires a rapid and accurate diagnosis through identification techniques. Identification can be based on Koch's Postulates combined with molecular techniques using Polymerase Chain Reaction (PCR).

Previous research has shown that *Rhizoctonia solani* exhibits high diversity. This diversity results in distinct morphological, genetic, nuclear, physiological, virulence, and hyphal anastomosis (AG) characteristics (Pralhad *et al*., 2019). Multinucleate *Rhizoctonia solani* is embedded in 14 distinct anastomosis groups (AGs), including AG-1 to AG-13 and AG-BI (bridging isolate). The AG1-IA anastomoses are generally found in corn and rice hosts, AG1-IB in lettuce hosts, AG1-1D in coffee hosts, AG3 in potato hosts, AG4 in potato and soybean hosts has a high degree of virulence, and AG2-2IIIB in soybean and beet hosts (Wallon *et al*., 2020).

Identification of *R. solani* using RAPD (Randomly Amplified Polymorphic DNA) yielded diverse phylogenetic relationships among isolates. Characterization of *R. solani* using the RAPD method in Egypt resulted in isolates 28, 31, 32, 33, and 34 belonging to the same anastomoses group, AG2-2. However, the primers used yielded low sensitivity, necessitating further sequencing (Mahmoud *et al*., 2012). Several studies have shown that *Rhizoctonia solani*, which infects corn, belongs to the same AG as rice. *R. solani* isolates from two different rice varieties were identified, with *R. solani* BA from rice, BNJ from corn, and NBR from corn belonging to the AG1-IA subgroup using specific primers Rs1F/Rs2R, characterized by brown to dark brown sclerotia on corn plants, and light brown on rice isolates (Bintang *et al*., 2017).

Resistance testing on corn plants after the identification process was conducted to predict the impact of sheath blight. The use of resistant corn varieties can influence the spread of the *R. solani* pathogen. Previous research showed that the average intensity of *R. solani* attacks on corn plants using the Pertiwi 5 corn variety was 10% and Bisi 959 was 11% in the mild category, and the NK 212, NK Perkasa, R7, Bisi 321, and Pertiwi 6 varieties were 23%, 13%, 13%, 21%, and 6% in the moderate category, respectively (Juliana *et al*., 2024).

Identification results can be used to assess *R. solani* morphology and conduct pathogenicity tests in corn inoculations by observing symptom variations in a short period of time and using a basic approach. Therefore, identifying *R. solani* is crucial for early disease detection. The aim of this study was to identify sheath blight disease in corn caused by *Rhizoctonia solani* and to examine the pathogenicity and virulence level of *Rhizoctonia solani* isolates from corn plants.

MATERIALS AND METHODS

Exploration of sheath blight symptoms. Exploration of corn sheath blight symptoms was conducted by searching for symptomatic plants in several different locations in Central Java, including Purwokerto in Sumbang District, Boyolali in Banyudono District, Klaten in Tulung District, Jepara in Batealit District, and Blora in Bogorejo District.

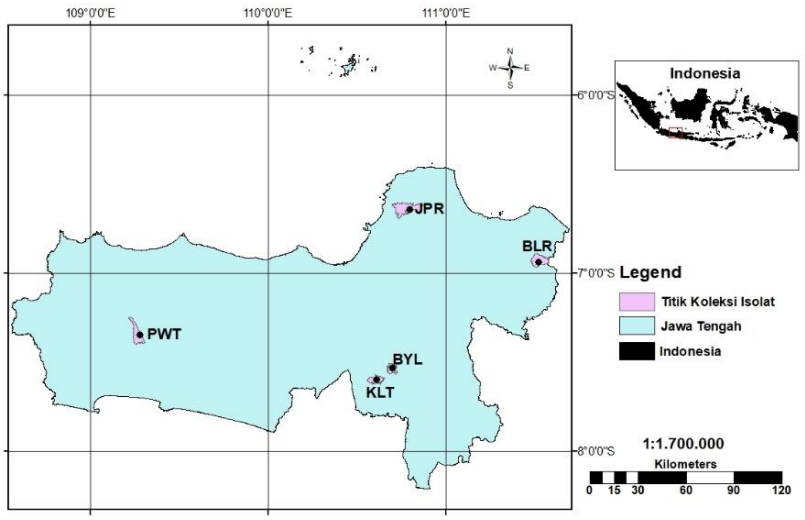

Fig 1_ Sampling Area of Corn Sheath Blight in Central Java, Indonesia

The sampling method used purposive sampling, which takes into account the incidence of sheath blight infecting corn plants. The observed symptoms included widespread tissue damage (necrosis) on the sheaths or leaves first or second from the ground, with irregular edges and the formation of white to brown sclerotium, causing the corn plants to rot, wilt, and develop chlorosis (Di *et al*., 2023).

Isolation of *Rhizoctonia solani*. Isolation of the pathogenic fungus *Rhizoctonia solani* was carried out by cutting parts of symptomatic and healthy plants measuring 0.5 cm x 0.5 cm. The parts of the plant affected by the fungus were sterilized using 70% alcohol for 1 minute and 0.5% NaOCl solution for 1 minute and rinsed with sterile distilled water and then dried. The specimen pieces were placed in Laminar Air Flow and then planted on PDA (Potato Dextrose Agar) media by making 39 grams of PDA media dissolved in 1000 ml of distilled water into a petri dish that had previously been sterilized. The petri dish was then labeled and incubated for 7 days at a temperature of approximately 28°C (Yadav *et al*., 2022).

Morphological identification of *Rhizoctonia solani*. The morphology of corn plant samples was observed macroscopically and microscopically. Macroscopic observation was carried out by growing *Rhizoctonia solani* isolates on PDA media and incubating them for 14 days. The characteristics, including sclerotia characteristics, namely the color and shape of the sclerotia, and mycelia characteristics, namely the color of the mycelia and the growth pattern of the mycelia, were observed. Microscopic observations were carried out using a light microscope to observe the length of the septum, diameter, number of nuclei, and branching angle of *R. solani*.

Molecular identification. Molecular identification of *Rhizoctonia solani* is performed using the PCR (Polymerase Chain Reaction) method. A master-mix of 12,5 µL reaction was prepared with the total volume 25 µL (master mix 12,5 µL; nuclease-free water 8,5 µL; primer forward and revers each 1 µL; and DNA sample 2 µL), Specific primer and PCR programs in the Table 1. Electrophoresis and visualization using 1% agarrose gel for 50 minutes at 50 volts. Amplification cycle program for specific primer AG1-IA, synthesis for 5 min at 94°C, denaturation for 30 s at 94°C, annealing for 30 s at 57°C, extension for 1 min at 72°C, final extension for 5 min at 72°C in 30 cyles. Specific primer

AG1-IB synthesis for 5 min at 95°C, denaturation for 15 s at 95°C, annealing for 15 s at 57°C, extension for 15 s at 72°C, final extension for 5 min at 72°C in 40 cyles. Specific primer AG2-2IIIB synthesis for 5 min at 94°C, denaturation for 1 min at 94°C, annealing for 1 min at 57,5°C, extension for 1 min at 72°C, final extension for 5 min at 72°C in 35 cyles

Primer	primary base sequence	Target (bp)	
Rs1F	5'-GCTTTTCTACCTTAATTTGGCAG-3'	137 – 140 (Sayler dan Yang, 2004)	Spesifik primer AG1 -IA
Rs2R	5'-GTGTGTAAATTAAGTAGACAGCAAATG-3'		
AG1 IB F	5'-TGGCCTTTTAACATTGGCATGT-3'	87 – 109 bp (Wallon *et al.,* 2020)	Spesifik primer AG1 IB
AG1 IB R	5'-CCAACCCCAAAGGACCTTGA-3'		
AG2-2 IIIB F	5'-AGGACGAGRCATGGATGGGAC-3'	322 – 330 (Salazar *et al.,* 2010)	Spesifik primer AG2-2 IIIB
AG2-2 IIIB R	5'-ACCTTGGCCAMCCTTTTTATC-3'		

Table 1. Primers, Base Sequences, and Targets Used for Identification of the Pathogen *Rhizoctonia solani*.

Pathogenicity Test. Pathogenicity tests were carried out at the Svarnaloka Indonesia field, Cepoko, Gunung Pati District, Semarang City. Starting from the preparation of the planting medium in a 35x35 cm polybag. NK212 corn seeds were soaked using a 1% NaOCl solution for 5 minutes and then planted. The inoculation stage was carried out using a completely randomized monofactor design with a control treatment without inoculation (IRS0), inoculum sources from Klaten (IRS1), Purwokerto (IRS2), Boyolali (IRS3), Jepara (IRS4), and Blora (IRS5). Mycelium pieces measuring 0.5x0.5 cm were taken from the planting medium and inserted into the sheath of the two corn plants aged 21 days after planting using aluminum foil and plastic wrap. The things observed include

a. the incubation period

b. diseases incidence (%) = DI = $\frac{n}{N}$ x 100%

c. diseases intensity (%) = DS = $\frac{\sum(nixvi)}{N \times V}$ x 100% using the following score: 0 no symptoms (0%), 1 blight occurs on the surface of the roots to the first leaf, turning light brown (1–25% light), 2 blight occurs from the roots to the third leaf sheath (26 – 50% moderate), 3 blight occurs from the third to sixth leaf sheath with –4 sclerotia appear (51 – 75% moderate heavy), 5 blight occurs throughout the plant, more than 5 sclerotia appear, and death occurs (76 – 100% heavy).

d. AUDPC (*Area Under the Disease Progress Curve*) value = $\sum_n^i (\frac{y_i+y_{i+1}}{2})\ t_{i+1} - t_i$

e. Growth rate infection = $r = \frac{1}{(t_2-t_1)} (\ln (\frac{x_2}{1-x_2}) - \ln (\frac{x_1}{1-x_1}))$

f. symptom variation. Observations of variations in symptoms that occur in plants are carried out every week starting from the germination phase until variations in symptoms appear.

g. plant height

h. number of leaves

i. number of cobs

j. and fresh weight per plant.

Statistical analysis. Qualitative data analysis from morphological observations of *Rhizoctonia solani* using Unweighted Pair Group Methode with Arithmetic mean (UPGMA) and quantitative analysis of pathogenicity tests using analysis of variance (ANOVA) test at 5% level and continued with Duncan Multiple Range Test (DMRT) test at 5% level.

Result

Exploration of sheath blight symptoms on maize.

Exploration of sheath blight symptoms in corn plants was carried out in five different areas.

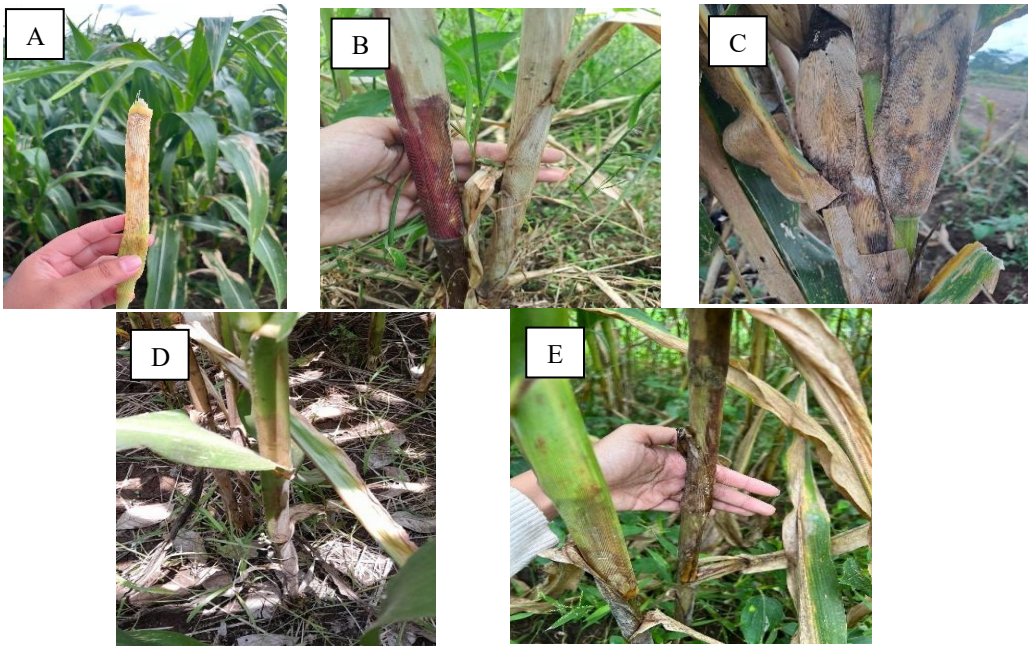

Fig 2_ Results of Plant Sample Exploration Based on Signs and Symptoms of Corn Sheath Blight. (A) Purwokerto (PWT), (B) Boyolali (BYL), (C) Klaten (KLT), (D) Jepara (JPR), (E) Blora (BLR)

Symptoms of corn plants caused by the *Rhizoctonia solani* pathogen, isolated from various regions, have been found to have similar signs and symptoms. Exploration of the symptoms of corn sheath blight caused by the *R. solani* pathogen revealed widespread tissue damage (necrosis) along the edges of the plant in an irregular manner, drying leaves, and plant death (Fig 1). Symptoms of corn plants infected with sheath blight include necrosis of the sheath tissue, forming an irregular, lesion-like pattern, causing the sheath to wilt (Di *et al*., 2023). Signs of *R. solani* infecting corn plants include the growth of sclerotia, a thick, white mass of mycelium that gradually changes color to blackish brown (Bintang *et al*., 2017).

The *Rhizoctonia solani* pathogen can spread both within and on the soil surface, so the plant parts most quickly infected are the sheath, roots, and lower stem. The *R. solani* pathogen is a type of soil-borne pathogen that can survive longer in the soil with a special defense structure in the form of sclerotium (Akber and Fang, 2024).

Morphological identification of *Rhizoctonia solani*

Identification of *Rhizoctonia solani* morphology microscopically was carried out using a light microscope at 40x10 magnification to determine morphometric characteristics in the form of septum length, hyphal diameter, number of nuclei, and hyphal branching angles and at 100x10 magnification to see the number of *Rhizoctonia solani* nuclei.

Isolates	Characteristics			
	Septum lenght	Diameter	Number of nuclei	Hyphal branching
	------- µm ---------			
Prwokerto (PWT)	45,41	6,21	6 (multinukleat)	90º
Boyolali (BYL)	79,56	4,51	8 (multinukleat)	90º
Klaten (KLT)	57,55	4,79	9 (multinukleat)	90º
Jepara (JPR)	71,13	5,65	9 (multinukleat)	90º
Blora (BLR)	76,15	4,58	10 (multinukleat)	90º

Table 2. Result of the length of the septum, diameter, number of nuclei, and branching angle of *Rhizoctonia solani*.

The variation of morphological characteristics of the five isolates resulted in varying septum lengths and diameters, the same number of nuclei, and had the same branching angle of 90º (Fig 2 and Fig 3). The morphometrics of the PWT isolates resulted in a septum length of 45.41 µm and a diameter of 6.21 µm, the BYL isolates resulted in a septum length of 79.56 µm and a diameter of 4.51 µm, the TLC isolates resulted in a septum length of 57.55 µm and a diameter of 4.79 µm, the JPR isolates resulted in a septum length of 71.13 µm and a diameter of 5.65 µm, and the BLR isolates resulted in a septum length of 76.15 µm and a diameter of 4.58 µm and the number of nuclei of the five isolates was included in the multinucleate group (Table 2). Based on these morphological and morphometric characteristics, the five isolates belong to the *Rhizoctonia solani* pathogen. The *Rhizoctonia solani* pathogen in corn plants has a septum length between 5.4 µm and 8.82 µm and a branching angle of 90° (Maddela *et al*., 2023).

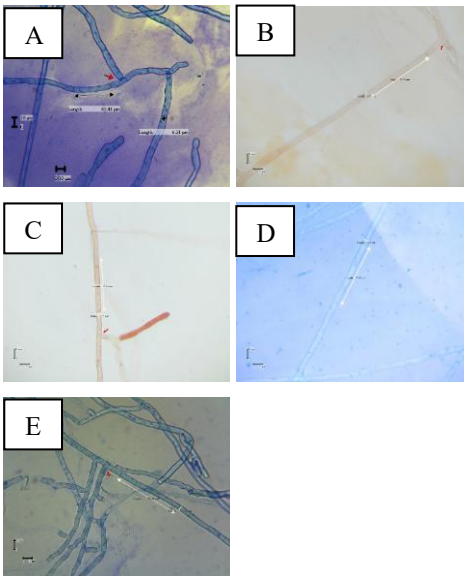

Fig 3_ Measurement of the length of the septum, hypha diameter, and branching angle of *Rhizoctonia solani*. (A) Isolate from PWT, (B) Isolate from BYL, (C) Isolate from TLC, (D) Isolate from JPR, (E) Isolate from BLR.

The septa of the pathogen *Rhizoctonia solani* are transverse lines within the hyphae and are generally located near the branching points between hyphae. The septa of the five *R. solani* isolates used in the pathogenicity test, as shown in Fig 2, are similar in shape but vary in length. The septa in *R. solani* serve as barriers between hyphae, thus protecting cell viability. The nutrient transport pathway in *R. solani*, which passes through the pores of the septa, aids fungal growth and development (Datta *et al*., 2021).

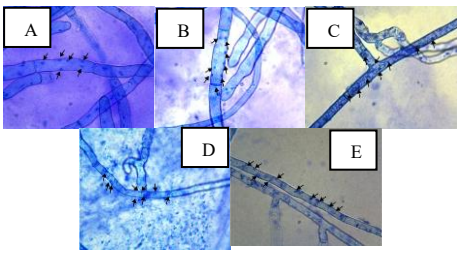

Fig 4_ Number of *Rhizoctonia solani* Nuclei. (A) Isolate from PWT, (B) Isolate from BYL, (C) Isolate from KLT, (D) Isolate from JPR, (E) Isolate from BLR.

The number of nuclei in the five Rhizoctonia solani isolates observed resulted in more than two nuclei, so they were categorized as multinucleate nuclei. The location of the number of nuclei in the fungus *R. solani* is inside the hyphae and separated by a hyphal partition and the nucleus is round. The number of nuclei can be seen in Fig 3. The multinucleate hyphae category in *R. solani* is classified as a pathogenic fungus. Mutinucleate hyphae have many nuclei so they can influence increased fungal metabolic activity, adaptation to the environment and higher pathogen virulence (Muzhinji *et al*., 2015). Some *R. solani* fungi which are categorized as binucleate hyphae can show a mutualistic symbiotic relationship. Binucleate *Rhizoctonia solani* can associate with plants to reduce the development of the *R. solani* pathogen, especially on plant roots (Taheri *et al*., 2024).

Macroscopic identification of the pathogenic fungus *Rhizoctonia solani* was carried out using the naked eye and the RHS (Royal Horticultural Society) color chart to

determine the diversity of sclerosia color characteristics, sclerosia distribution patterns, mycelium color and mycelium growth patterns. Macroscopic observation results using isolates aged 14 DAI (Days After Isolation) in .

Isolates	Characteristics			
	Sclerotia color	Sclerotia distribution pattern	Mycelia color	Mycelia distribution pattern
PWT	*Dark Greyish Yellowish Brown*	*Peripheral*	*Yellowish White*	*Scarce*
BYL	*Dark Greyish Yellowish Brown*	*Scattered*	*Palle Yellow*	*Scarce*
KLT	*Dark Greyish Reddish Brown*	*Peripheral*	*Yellowish White*	*Scarce*
JPR	*Dark Greyish Yellowish Brown*	*Scattered*	*Palle Yellow*	*Scarce*
BLR	*Dark Greyish Reddish Brown*	*Scattered*	*Palle Yellow*	*Scarce*

Table 3. Characteristics of Macroscopic Observation Results of *Rhizoctonia solani*

The diversity of macroscopic characteristics in Table 3. It is known that three isolates (PWT, BYL, JPR) have the same sclerotia color, namely Dark Greyish Yellowish Brown and two isolates (KLT and BLR) have Dark Greyish Reddish Brown sclerotia color. The characteristics of the sclerotia distribution pattern and mycelial color in PWT and KLT isolates have the same character, namely growing peripheral and yellowish white color and isolates BYL, JPR, and BLR have the character of growing scattered and pale yellow. The characteristics of the mycelial distribution pattern of *R. solani* five isolates in the same group are scarce. According to research by Maddela *et al*. (2023) it resulted that the growth pattern of *R. solani* sclerotia on corn plants varies, including in the central, on the peripheral, and scattered and the color of the sclerotia ranges from light brown to dark brown.

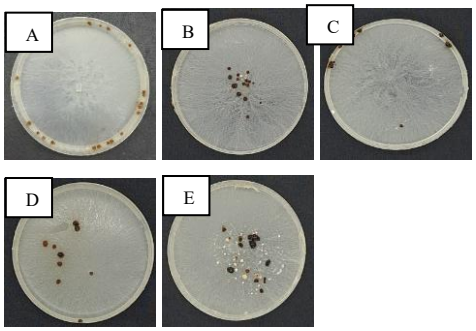

Fig 5_ Macroscopic Observation of the Front View of *Rhizoctonia solani*. (A) Isolate from PWT, (B) Isolate from BYL, (C) Isolate from TLC, (D) Isolate from JPR, (E) Isolate from BLR.

Observations show that isolates from each region have varying characteristics. The shape of *Rhizoctonia solani* sclerotia, as seen in Fig 4, is a collection of thickened, spherical hyphae that are white at the beginning of growth and darken as they age. Differences in sclerotia growth patterns, sclerotia color, and mycelial growth patterns can be influenced by environmental conditions such as temperature, pH, and light duration. *R. solani* isolate colonies can grow at temperatures ranging from 10 to 30°C with a pH ranging from 5 to 9, and grow optimally in the dark (Budiarti and Bintang, 2022).

Microscopic and macroscopic observations were regrouped to differentiate the character types of each isolate based on their kinship level using the Unweighted Pair-Groups Method using Arithmetic Averages (UPGMA). Based on the UPGMA analysis, the *Rhizoctonia solani* isolates produced two clusters that differentiated them based on their morphological characteristics.

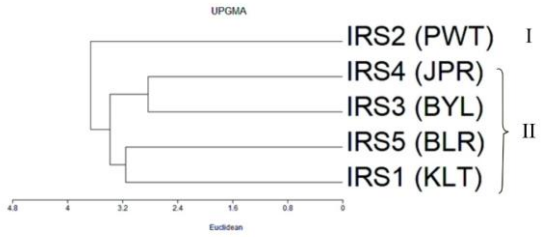

Fig 6_ Dendogram of *Rhizoctonia solani* isolates based on morphological characters using the UPGMA method. (PWT) isolates from Purwokerto, (JPR) isolates from Jepara, (BYL) isolates from Boyolali, (BLR) isolates from Blora, and (TLC) isolates from Klaten.

The UPGMA results show that 5 isolates were formed into 2 clusters, cluster 1 contained 1 isolate and cluster 2 consisted of 4 isolates (Fig 5). Cluster 1 is IRS2 isolate from Purwokerto. Cluster 2 is grouped into 2 subclusters including the first subcluster IRS4 isolate from Jepara and IRS3 isolate from Boyolali and the second subcluster IRS5 isolate from Blora and IRS1 isolate from Klaten. The distinguishing character in cluster 1 (PWT) and 2 (JPR, BYL, BLR, and TLC) is the color character of the front surface of the fungal mycelia. The distinguishing character between the 2 subclusters in the 2nd cluster (JPR, BYL and BLR, TLC) is the color of the fungal mycelia plate. The distinguishing characters of each isolate are the number of nuclei, diameter, and length of the septum. The level of similarity of the characters of the JPR, BYL, BLR, and TLC isolates is higher than that of the PWT isolate. The diversity of multinucleate *R. solani* pathogens has a high level of heterozogosity, causing genetic diversity even within one anastomosis group (Francis *et al*., 2023).

Moleculer identification of *Rhizoctonia solani*

The use of molecular identification using specific primers produces DNA bands that are used to identify the anastomosis of the *Rhizoctonia solani* group.

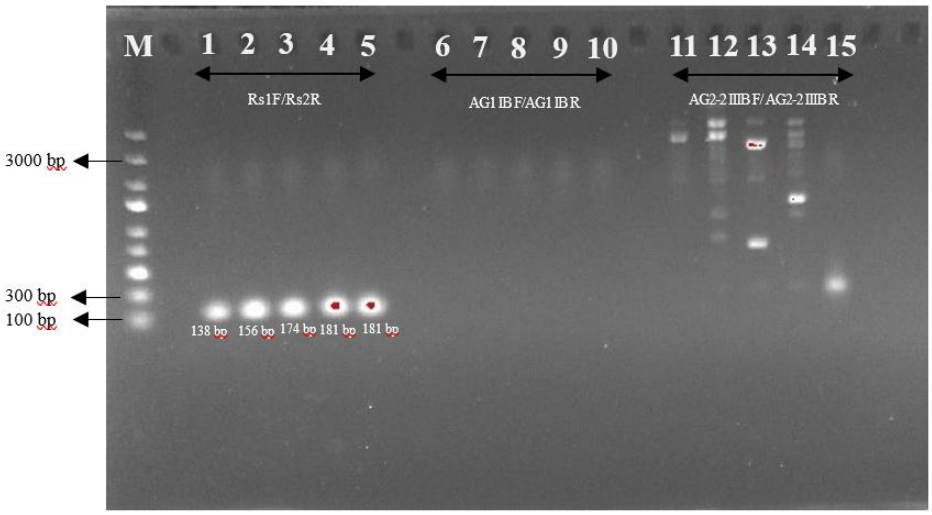

Fig 7_ Visualization of DNA Bands from Amplification Results on 1% Agarose Gel. (M) 100bp DNA marker (ThermoSci), (1) Primer Rs1F/Rs2R of JPR sample, (2) Primer Rs1F/Rs2R of BYL sample, (3) Primer Rs1F/Rs2R of PWT sample, (4) Primer Rs1F/Rs2R of KLT sample, (5) Primer Rs1F/Rs2R of BLR sample, (6) Primer AG1 IB F/AG1 IB R of JPR sample, (7) Primer AG1 IB F/AG1 IB R BYL sample, (8) Primer AG1 IB F/AG1 IB R PWT sample, (9) Primer AG1 IB F/AG1 IB R KLT sample, (10) Primer AG1 IB F/AG1 IB R BLR sample, (11) Primer AG2-2 IIIB F/AG2-2 IIIB R JPR sample, (12) Primer AG2-2 IIIB F/AG2-2 IIIB R BYL sample, (13) AG2-2 IIIB primer F/AG2-2 IIIB R PWT sample, (14) AG2-2 IIIB primer F/AG2-2 IIIB R TLC sample, (15) AG2-2 IIIB primer F/AG2-2 IIIB R BLR sample.

The five samples identified macroscopically and microscopically were subjected to molecular testing using specific primers. The results of DNA visualization were that the five samples from IRS4 (JPR), IRS3 (BYL), IRS2 (PWT), IRS1 (TLC), and IRS5 (BLR) identified using specific primers Rs1F/Rs2R produced DNA bands with sizes of 138 bp, 156 bp, 174 bp, 181 bp, and 181 bp, respectively (Fig. 6) can be grouped into *Rhizoctonia solani* anastomosis group AG1 1A. The use of specific primers Rs1F/Rs2R to detect *Rhizoctonia solani* pathogens in corn plants included in the anastomosis group AG1-1A isolates from Jepara with a size of 140 bp (Bintang *et al*., 2017). Visualization results using specific primers AG1 IB F/AG1 IB R did not produce DNA bands, thus excluding them from the AG1 IB anastomosis group.

Visualization results using specific primers AG2-2 IIIB F/AG2-2 IIIB R produced random DNA bands, excluding those belonging to the *R. solani* anastomosis group AG2-2 IIIB. DNA visualization using specific primers AG2-2 IIIB detects anastomoses in this group at a size of 322–330 bp (Salazar *et al*., 2000). Visualization using specific primers AG2-2 IIIB F/AG2-2 IIIB R detected random bands due to the distant kinship between the AG1-1A and AG2-2 IIIB anastomoses. Gene evolution occurred in the anastomosis of the AG1-1A group with the duplicated AG2 – 2IIIB so that a kinship was detected in the two anastomoses of the groups (Francais *et al*., 2023).

Phatogenicity Test

Incubation period

The incubation period of the *Rhizoctonia solani* pathogen indicates the time period required for the pathogen to infect the plant until the first symptoms appear.

Inoculum Sources	Incubation period
	---------- Days ----------
IRS0 (without inoculum)	-
IRS1 (KLT)	7,60
IRS2 (PWT)	8,45
IRS3 (BYL)	5,80
IRS4 (JPR)	7,50
IRS5 (BLR)	5,80

Table 4. Incubation Period of *Rhizoctonia solani* on Corn Plants with Inoculation Treatment from Different Inoculum.

The incubation period of *Rhizoctonia solani* pathogen inoculated on corn plants with different inoculum source treatments resulted in IRS0 treatment without inoculation did not show any variation in sheath blight symptoms due to the pathogen, IRS1 treatment of isolates from Klaten showed variations in initial symptoms on average day 7.6, IRS2 treatment of isolates from Purwokerto showed variations in initial symptoms on average day 8.45, IRS3 treatment of isolates from Boyolali and IRS5 treatment of isolates from Blora showed variations in initial symptoms on average day 5.80, and IRS4 treatment of isolates from Jepara showed variations in initial symptoms on average day 7.50. The incubation period of 5-8 days in the pathogenicity test is still appropriate to indicate the time period between the pathogen infecting the plant until the first disease symptoms appear. The incubation period for *Rhizoctonia solani* occurs from 7 to 16 days after inoculation, or 26 days after planting (Tampubolon *et al*., 2026).

The treatment without inoculum showed no variation in initial symptoms because *R. solani* is a soil-borne pathogen. The soil-borne pathogen *R. solani* can infect plants because it possesses a resistant structure in the form of sclerotia that can survive and spread through the soil, especially in roots and plant tissues in direct contact with the soil (Manasikana

et al., 2021). *Rhizoctonia solani* isolates from Boyolali and Blora produced the fastest initial symptoms, starting on day 5, and the isolate from Purwokerto produced the slowest initial symptoms, starting on day 8 (Table x). Factors influencing the speed of initial symptoms were the average temperature in the test field, which was 32°C. The suitable environmental temperature for the development of *R. solani* is 30°C (Budiarti and Bintang, 2022).

Diseases Incidence

The diseases incidence aims to determine the percentage of the number of plants attacked by leaf blight disease caused by *Rhizoctonia solani*.

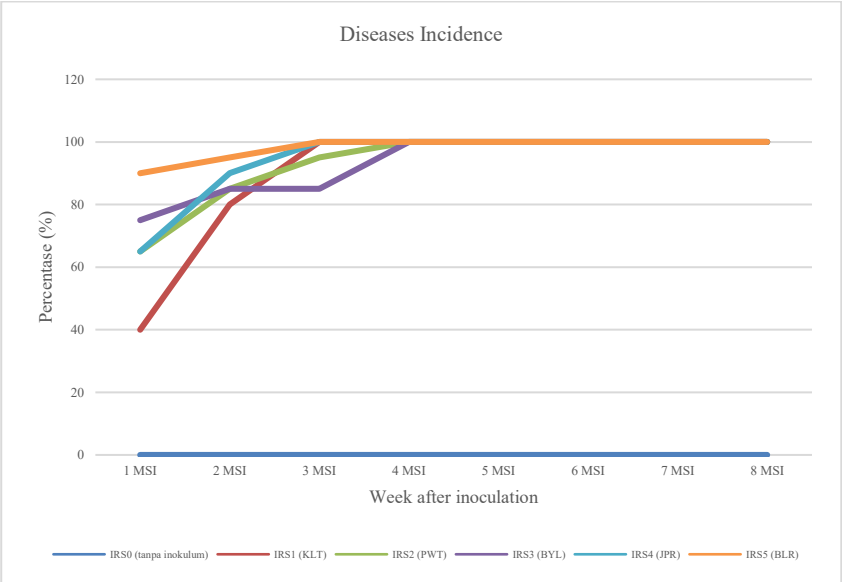

Fig 8_ Incidence of Sheath Blight Disease in Corn Plants with Inoculation Treatments from Different Inoculum.

The incidence of sheath blight caused by *Rhizoctonia solani* inoculated into corn plants with different inoculum treatments increased weekly, starting from 40–90% in the first week, 80–95% in the second week, 85–100% in the third week, and 100% in the fifth to eighth weeks (Fig 7). Treatment without inoculum resulted in a disease incidence of 0% because the plants were not affected by sheath blight and there was no variation in symptoms caused by the *R. solani* fungus. This is because *R. solani* is a soil-borne pathogen that spreads through direct contact with the soil (Akber and Fang, 2024).

The inoculation treatment of plants with different isolate origins resulted in the lowest incidence rate in the first week at 40% and the highest in the third week and thereafter at 100%, which means that all tested plants experienced symptoms due to *R. solani* such as leaf sheaths experiencing widespread and uneven tissue damage and signs of sclerotia that appeared in the fourth week after incubation. The high incidence rate occurred because the NK 212 corn variety planted was included in the category susceptible to sheath blight disease. The use of the NK 212 corn variety resulted in the highest intensity of *R. solani* attacks (Juliana *et al*., 2024).

Diseases Intensity

Plant disease intensity is used to indicate the severity of disease in a corn plant population.

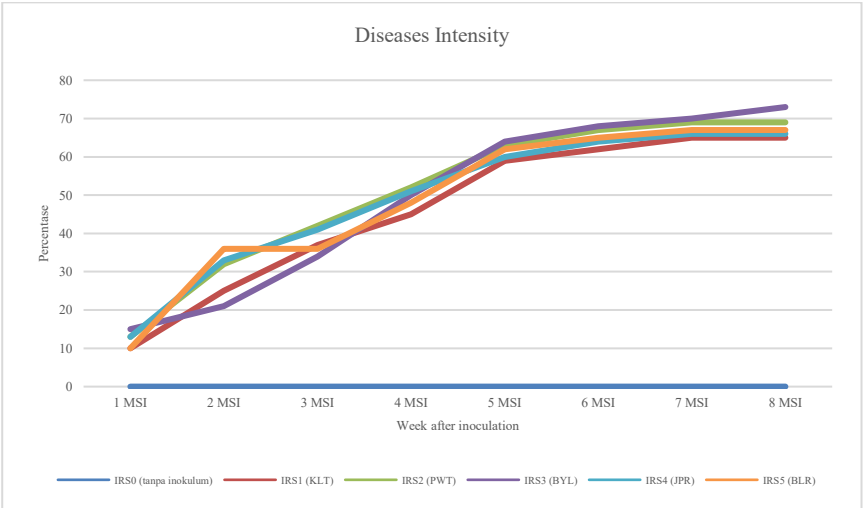

Fig 9_ Intensity of Sheath Blight Disease in Corn Plants with Inoculation Treatments from Different Inoculum.

The intensity of sheath blight disease caused by *Rhizoctonia solani* based on different inoculum source treatments increased every week except for the treatment without inoculum. The treatment without inoculum resulted in an attack intensity of 0% due to the absence of symptoms and signs caused by the *R. solani* pathogen. An attack intensity value of 0% indicates the absence of corn sheath blight disease and healthy plants (Shi *et al*., 2021). The inoculum source treatments at 8 WAS produced the highest attack intensity, namely treatment IRS3 (BYL) at 73%, followed by IRS2 (PWT) at 69%, IRS5 (BLR) at 67%, IRS4 (JPR) at 66%, and IRS1 (KLT) at 65% in the moderately severe category (Fig 8). Environmental conditions in the experimental field with an average daily temperature of 32 °C support the development of sheath blight disease caused by the *R. solani* pathogen, thus affecting the level of disease intensity. The optimum temperature for *R. solani* to thrive is 30°C, with sclerotia developing at 11–37°C (Di *et al*., 2023).

The highest attack intensity, at 73%, was categorized as moderately severe. Symptoms and signs of sheath blight include damage to plant tissue caused by *R. solani* in the moderately severe category, ranging from the third to sixth sheath, with an attack intensity score of 50–80% (Hamidi *et al*., 2024). High attack intensity is influenced by susceptible hosts. Susceptible hosts in corn can be identified by increasing AUDPC values ranging from 240–685 (Muis *et al*., 2022). Plant age is a contributing factor to increased disease intensity. The *R. solani* pathogen develops sclerotia during the late vegetative phase. Sclerotia formation occurs at 60 days after planting or 30 days after inoculation in corn plants (Hamidi *et al*., 2024).

The Area Under the Disease Progress Curve (AUDPC) Value

The Area Under the Disease Progress Curve (AUDPC) value indicates the resistance of corn plants to sheath blight disease.

Inoculum Sources	AUDPC values	Plant Resistance Category
IRS1 (KLT)	330,50	Moderate
IRS2 (PWT)	366,00	Moderate
IRS3 (BYL)	351,00	Moderate
IRS4 (JPR)	354,50	Moderate
IRS5 (BLR)	352,50	Moderate

Table 5. AUDPC Value of *Rhizoctonia solani* on Corn Plants with Inoculation Treatment from Different Inoculum.

The AUDPC value for the IRS2 (PWT) treatment, an isolate from Purwokerto, produced the highest value at 366.00, followed by the other treatments: IRS4 (JPR) at 354.50, IRS5 (BLR) at 352.50, IRS3 (BYL) at 351.00, and IRS1 (KLT) at 330.50, with the same plant category: susceptible. The AUDPC value is based on the observed disease intensity. Disease intensity at the end of the observation period is categorized as moderately severe, indicating the plant has a low level of resistance to the inoculated pathogen, or susceptible. AUDPC values for corn plants affected by sheath blight of 240–685 are considered susceptible (Muis *et al*., 2022).

The AUDPC value is used to describe the level of plant resistance based on observed disease intensity over time. Corn sheath blight disease intensity in the moderately severe category indicates a susceptible host. The higher the AUDPC value, the more susceptible the corn plant is to sheath blight. Conversely, the lower the AUDPC value, the more resistant the plant is to sheath blight (Muis *et al*., 2022). The AUDPC value can be influenced by the severity of the disease affecting the corn plant. A disease severity percentage of 50–80% is considered susceptible, and 90–100% is considered highly susceptible (Hamidi *et al*., 2024).

Growth Rate Infection

The disease infection rate indicates the speed of spread of leaf blight disease caused by pathogens in a unit of time.

Inoculum sources	Growth rate infection values (r)							
	1 WAI	2 WAI	3 WAI	4 WAI	5 WAI	6 WAI	7 WAI	8 WAI
	unit/week							
IRS0 (without inoculum)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
IRS1 (KLT)	0,00	1,10	0,57	0,33	0,56	0,12	0,13	0,00
IRS2 (PWT)	0,00	1,15	0,43	0,40	0,45	0,17	0,09	0,00
IRS3 (BYL)	0,00	0,41	0,66	0,66	0,57	0,18	0,09	0,15
IRS4 (JPR)	0,00	1,19	0,34	0,40	0,36	0,17	0,09	0,00
IRS5 (BLR)	0,00	1,62	0,00	0,49	0,57	0,13	0,09	0,00

Table 6. Growth Rate Infection Value of *Rhizoctonia solani* on Corn Plants with Inoculation Treatment from Different Inoculum.

The infection rate of corn sheath blight at 1 week after incubation (WAI) was 0.00 plants/week. The highest infection rates were found in treatments IRS1, IRS2, IRS4, and IRS5, respectively, at 1.10; 1.15; 1.19; and 1.62 plants/week at 2 WAI, and in treatment IRS3 at 0.66 plants/week at 4 WAI, with a decrease thereafter. A high infection rate indicates the pathogen can develop and infect plants, while a low infection rate indicates a decrease in plant infection due to *Rhizoctonia solani* inoculation (Tampubolon *et al*., 2026).

The inoculated *Rhizoctonia solani* isolates had high virulence, a suitable environment, and susceptible host conditions, resulting in higher disease intensity at 2 WAI compared to subsequent observation periods. The infection rate of the *R. solani* pathogen, which belongs to the monocyclic pathogen group, tends to increase in population at the beginning of inoculation and has a slow death rate. The infection rate of the disease caused by the *Rhizoctonia solani* pathogen is more dynamic due to suitable environmental conditions of temperature and humidity, so that infection occurs more quickly (Tampubolon *et al*., 2026). One factor influencing the infection rate is the condition of the susceptible host, as seen in the AUDPC value category of plant resistance (Table 5). A high AUDPC value makes corn plants susceptible to sheath blight, conversely, a lower AUDPC value makes the plant resistant to sheath blight (Muis *et al*., 2022).

Symptom Variation

Symptom variation is the difference in the form of signs and symptoms that occur in corn plants inoculated with the *Rhizoctonia solani* pathogen.

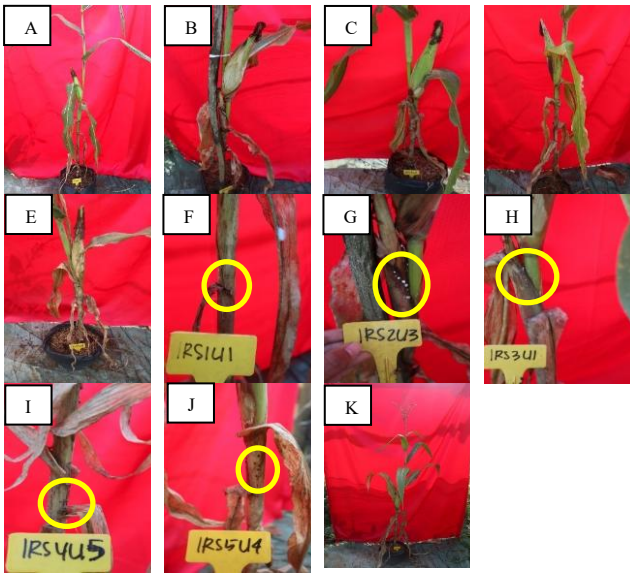

Fig 10_ Documentation of Symptom Variation at 8 WAI. (A) Symptoms of Isolates from TLC, (B) Symptoms of Isolates from PWT, (C) Symptoms of Isolates from BYL, (D) Symptoms of Isolates from JPR, (E) Isolates from BLR, (F) Sclerosia of Isolates from TLC, (G) Sclerosia of Isolates from PWT, (H) Sclerosia of Isolates from BYL, (I) Sclerosia of Isolates from JPR, (J) Sclerosia of Isolates from BLR, (K) Control plants affected by bacterial wilt.

Variations in symptoms occur in plants inoculated by the *Rhizoctonia solani* pathogen starting from the age of 2 Post Incubation Periods (WAI) to 8 WAI. Variations in symptoms that form at the age of 2-3 WAI are the appearance of sheath blight symptoms from the lower stem of the plant in the form of oval lesions resulting in tissue damage to the corn sheath and the sheath turning yellow as shown in Fig 9, Figure E. Symptoms of sheath blight caused by the *R. solani* pathogen that occurs at the age of 33 DAI are infection up to the second sheath of the corn plant and the lesions enlarge with dark edges (Tampubolon *et al*., 2026). Variations in symptoms that occur at the age of 6-8 WAI enter the generative phase of cob formation, namely the corn husk is white and there are widespread spots (Fig 9). Corn kernel formation is uneven and the outer skin of the corn fruit is white and there are lesions that merge (Hamidi *et al*., 2024). Plant sclerotia form during the late vegetative phase and the generative phase, at 4–8 days after planting (WAS), as indicated by the yellow circle. Sclerotia develop a white color for 3–4 days, then gradually change color to brown as the sclerotia mature. Sclerotia act as a protective structure for the *R. solani* pathogen, forming thickened and hardened hyphae that allow it to grow even under unfavorable conditions (Moni *et al*., 2016). Symptoms are influenced by suitable environmental conditions and a susceptible host, allowing the pathogen to infect the plant. Susceptible hosts and high humidity can influence the appearance of variations in *R. solani* symptoms (Tampubolon *et al*., 2026).

Symptoms affecting corn plants in the control treatment in the field for bacterial wilt include parallel pale green-yellowish lines along the leaf veins, and wilting and drying of the leaves. Based on these symptoms, corn wilt is suspected to be caused by the bacterium *Pantoea stewartii* (Temaja *et al*., 2017). High rainfall conditions allow bacteria to thrive due to the favorable environment. The optimal temperature for bacterial wilt disease development in corn plants is 27–30°C (Djaenuddin and Muis, 2018).

Plant Height and Number of leaves

Plant height and number of leaves in corn plants inoculated with the pathogen *R. solani* with different inoculum source treatments can be seen in Table 7.

Inoculum sources	Plant Height	Number of Leaves
	------- cm -----	
IRS0 (without inoculum)	133,75	7,80
IRS1 (KLT)	151,10	8,75
IRS2 (PWT)	146,05	8,45
IRS3 (BYL)	125,75	7,40
IRS4 (JPR)	145,00	8,70
IRS5 (BLR)	127,65	6,85

Table 7. Plant Height and Number of Leaves in Corn Plants Inoculated with *Rhizoctonia solani* with Different Inoculum Source Treatments

The height of corn plants inoculated with *Rhizoctonia solani* using inoculum sources from different regions at 11 weeks after planting (WAP) showed no significant differences based on analysis of variance. The highest plant height was in the IRS1 treatment at 151.10 cm, followed by IRS2 at 146.05 cm, IRS4 at 145.00 cm, IRS0 at 133.75 cm, IRS5 at 127.65 cm, and the lowest was in the IRS3 treatment at 125.75 cm (Table 7). These results may occur because the inoculated *R. solani* did not affect the plant growth phase, especially in the vegetative phase. The height of corn plants planted in polybags at week 8 was 105–160 cm (Prakoso *et al*., 2022). Based on the description of the NK 212 corn variety, the height of the corn plants does not meet the standard variety description, so inoculation of corn plants with *R. solani* can affect plant height. The height of corn plants inoculated with the inoculum source from Klaten produced the highest plants at 151.10 cm, and the lowest with the inoculum source from Boyolali at 125.75 cm. The difference in plant height indicates that the pathogen inoculation can infect plant tissue and that the isolate has a high degree of virulence. High virulence can influence the intensity of sheath blight attacks caused by *Rhizoctonia solani* (Tampubolon *et al*., 2026).

The number of leaves in corn plants inoculated with *Rhizoctonia solani* using inoculum sources from different regions at 11 weeks after planting (WAP) showed no significant difference based on analysis of variance. The lowest average number of leaves was 6.85 in the Blora inoculum treatment, while the highest was 8.75 in the Klaten inoculum treatment. This indicates that the number of leaves is still lower than that of normally growing corn plants because the *R. solani* pathogen infects the plant's leaf tissue, reducing leaf quality and quantity. The average number of leaves on corn plants is 13–14 at 7 weeks after planting (WAP) (Prakoso *et al*., 2022).

Fresh Weight per Plant and Number of Cobs

Fresh weight per plant and number of cobs in corn plants inoculated with the pathogen *R. solani* with different inoculum source treatments can be seen in Table x.

Inoculum sources	Fresh Weight	Number of Cobs
	--- gram/plant --	
IRS0 (without inoculum)	305,50	0,70
IRS1 (KLT)	313,80	0,85
IRS2 (PWT)	291,20	0,55
IRS3 (BYL)	255,00	0,75
IRS4 (JPR)	289,25	0,60
IRS5 (BLR)	238,25	0,75

Table 8. Plant Fresh Weight and Number of Cobs in Corn Plants Inoculated with *Rhizoctonia solani* with Different Inoculum Source Treatments

The fresh weight of corn plants inoculated with *Rhizoctonia solani* using inoculum sources from different regions at 11 weeks after planting (WAP) showed no significant differences based on analysis of variance. The highest fresh weight was obtained from the Klaten inoculum source, at 313.80 grams per plant, and the lowest was obtained from Blora, at 238.25 grams per plant. The average fresh weight of normal corn is more than 200 grams per plant (Nurhayati *et al*., 2022). Corn plant weight is considered normal because the level of pathogen attack has not significantly inhibited plant biomass accumulation, allowing water absorption and vegetative growth to proceed normally. Corn plants are attacked by sheath blight disease with the highest intensity in the late vegetative phase, allowing plants to grow normally (Tampubolon *et al*., 2026).

The number of corn cobs inoculated with *Rhizoctonia solani* using inoculum sources from different regions at 11 weeks after planting (WAP) based on analysis of variance showed no significant differences. The highest average number of cobs was found in the Klaten inoculum source at 0.85 and the lowest in the Purwokerto inoculum source at 0.55. The average number of cobs in healthy corn plants is 1 cob per plant (Utami *et al*., 2022). The results showed that the number of cobs was lower because the pathogen infection in the plant during the generative phase was greater, disrupting the physiological activity of the leaves for corn fruit formation. *Rhizoctonia solani* attacks in the late vegetative phase with symptoms of drying leaves and whitening of the cob husks (Tampubolon *et al*., 2026).

Conclusion:

Based on the research results, it can be concluded that the identification of the pathogen causing sheath blight in corn plants is *Rhizoctonia solani* based on macroscopic and microscopic observations as well as molecular identification resulting in *Rhizoctonia solani* anastomosis group AG1-1A. Isolates from Boyolali showed the fastest incubation period and the highest disease intensity compared to other isolates. The results of the pathogenicity test showed a diversity of virulence levels among the five inoculum sources used.

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