

# Resistance selection of local sorghum varieties in East Nusa Tenggara, Indonesia against *Rhizoctonia solani*

EWINDA ISENSI FENI, SRI KASMIYATI\*, V. IRENE MEITINIARTI

Department of Biology, Faculty of Biology, Universitas Kristen Satya Wacana. Jl. Diponegoro No. 52-62, Salatiga 50711, Central Java, Indonesia.  
Tel./Fax.: +6285640612428, \*email: kas@uksw.edu

Manuscript received: 3 July 2023. Revision accepted: 13 October 2023.

**Abstract.** Feni EI, Kasmiyati S, Meitiniarti VI. 2023. Resistance selection of local sorghum varieties in East Nusa Tenggara, Indonesia against *Rhizoctonia solani*. *Biodiversitas* 24: 5309-5318. Sorghum is a multifunctional crop that can be used as an alternative food for the people of East Nusa Tenggara. However, one of the challenges in cultivating sorghum in this region is the presence of the *Rhizoctonia solani* pathogen. This fungus causes a devastating root rot disease in sorghum crops, resulting in loss of crop yields and potentially disrupting food security in the region. Using resistant varieties is one of the most effective ways to prevent the growth of *R. solani* biologically. Therefore, it is important to study the resistance of local sorghum varieties. The purpose of this study was to determine different sorghum varieties' resistance responses to the *R. solani* fungus that causes root rot disease. This study used a completely randomized design (CRD) with 2 levels of treatment: the treatment of sorghum varieties and the treatment of fungi inoculums, which were inoculated separately and simultaneously. Each treatment was repeated 3 times. Observations and data collection were carried out on the 7<sup>th</sup> day, and some of the parameters observed included germination percentage, radicle length, coleoptile length, sprout length, GTI value, and disease incidence value. The sorghum variety with the highest resistance value was found in the red variety. This red variety had a disease incidence value of 63.3%, much lower than the other four test varieties. These results provide important insights for developing a more root rot disease-resistant local sorghum plant variety.

**Keywords:** *Rhizoctonia solani*, root rot, sorghum, varietal resistance

## INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is Indonesia's third food cereal crop after rice and corn. Sorghum has considerable potential to be developed as a food source that is tolerant of drought and inundation and can adapt to less fertile land (Sulistiyawati et al. 2019; Thunay et al. 2019). Sorghum is known to have nutritional content that is not inferior to other cereals, containing functional food elements such as carbohydrates (70.24-77.52%), protein (9.03-12.73%), and fat (3.28%) (Mukkun et al. 2021). This indicates that sorghum can be used as an alternative food in responding to the needs of some communities, in line with regional developments and rapid population growth. Besides its potential as a food ingredient, sorghum is a multifunctional plant because it can be used as an ingredient in animal feed, industrial raw materials, and an export commodity (Zubair 2018; Paiva et al. 2022). Even though the potential for sorghum is very large, the productivity of sorghum crops in Indonesia, especially in East Nusa Tenggara, is still low, ranging from 0.55 tons to 1.10 tons per hectare (Lestari et al. 2019).

One of the obstacles to low local sorghum production is farmers' lack of knowledge regarding how to control root rot disease caused by the fungus *R. solani*. Based on the study of Rusae et al. (2018), the resistance of local sorghum varieties to *R. solani* fungus is still low. *Rhizoctonia solani* fungus is known as a soil-borne pathogen that has a wide host range and causes disease in

more than 200 species of plants, including cereal crops such as rice, corn, sorghum, wheat, barley, and beans (Shi et al. 2020; Basbagci et al. 2019). Based on Suharti et al. (2023) and Koch et al. (2022), *R. solani* can cause sheath blight, leaf blight, damping-off, and root rot. The level of attack by *R. solani* on sorghum plants ranges from 8.82 to 40% (the attack level is different for each variety) (Nafriana et al. 2013). Apart from that, *R. solani* is also an agent that causes sheath blight in rice plants, which can cause damage to rice plants and reduce rice yields by up to 50-80% (Dewi et al. 2020).

Transmission of root rot caused by *R. solani* can occur through direct contact with infected plant roots or seeds infected with this pathogenic fungus. The *R. solani* fungus can survive in infected plant tissue in active (mycelium) and dormant (sclerotia) forms for years (Xue et al. 2018). This causes root rot caused by *R. solani* to be relatively difficult to control. If it is not handled properly, the productivity of vulnerable local sorghum varieties will continue to decline and lead to extinction.

Therefore, one effort to biologically control root rot disease caused by the fungus *R. solani* is to use local sorghum varieties resistant to this pathogen. This variety has good genetics, which helps reduce the risk of fungal infections, maintain plant productivity, and maintain environmental balance. Plant genetics plays an important role in controlling disease caused by the *R. solani* pathogen (Tesema et al. 2022). For example, the research conducted by Rao et al. (2020), on rice varieties demonstrated that

genetic variations among different varieties significantly influence the level of resistance and susceptibility to diseases caused by *R. solani*. By understanding these genetic factors, research can be directed to determine different resistance responses of each local sorghum variety to diseases caused by the *R. solani* fungus. Based on research by Koima et al. (2023) and Kumi et al. (2019), using resistant sorghum genotypes is one of the most efficient, cost-effective, and environmentally friendly strategies in reducing the rate of development of plant diseases caused by pathogens.

Thus, it is necessary to test the resistance of local sorghum varieties to the fungus *R. solani*. The aim is to determine different sorghum varieties' resistance responses to the *R. solani* fungus, which causes root rot diseases. This step is crucial because it recommends that farmers develop and use local sorghum varieties that are more resistant to root rot disease caused by *R. solani*, thereby reducing the risk of losses due to disease and increasing crop yields.

## MATERIALS AND METHODS

### Test plants and *Rhizoctonia solani* culture preparation

The tested plants used in this study were local sorghum with 5 varieties. Seeds of local sorghum (*Sorghum bicolor* (L.) Moench) were obtained from Malaka Regency, East Nusa Tenggara Province. The 5 local sorghum varieties were marked with different colors: white, yellow, red, blackish red, and black. The pure culture of *R. solani* was obtained from the culture collection of the Laboratory of Plant Diseases, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia, with access number MT367900, which has been deposited with the National Center for Biotechnology Information (NCBI). The culture of *R. solani* was maintained in a Potato Dextrose Agar (Merck) slant and incubated in an incubator at 29-32°C. Cultures were sub-cultured every two weeks.

### Procedures

Testing sorghum varieties' resistance differences was conducted at the Microbiology Laboratory, Faculty of Biology, Satya Wacana Christian University, Salatiga, Indonesia. In this test, 2 levels of treatment were used. First, local NTT sorghum varieties were inoculated simultaneously with the *R. solani* fungus. Second, local NTT sorghum varieties were separately inoculated with the *R. solani* fungus. The test for resistance differences of the sorghum varieties was prepared using a completely randomized design (CRD), which consisted of 2 treatment levels. Each treatment was repeated 3 times. These following steps are taken in testing the resistance response differences of the sorghum varieties to the *R. solani* fungus, including:

#### Production of *Rhizoctonia solani* inoculum

One ose of *R. solani* sclerotium was grown into PDA slant media and incubated for 7-10 days until brown sclerotia turned blackish. Sclerotia is ready to be inoculated into sorghum plants.

#### Preparation of sorghum seeds

Prepared sorghum seeds of each variety and surface sterilized by soaking in 1% sodium hypochlorite (commercial) solution for 1 minute and rinsed the seeds with sterile distilled water three times to remove sterility. The sorghum seeds were soaked in sterile distilled water for 24 hours to break the dormancy period. After the soaking period, the seeds are air-dried. Ten healthy sorghum seeds (each variety) of relatively the same size were selected and used as test plants.

#### Resistance test of sorghum variety to *Rhizoctonia solani*

**Separate inoculation.** Firstly, a PDA petri dish was prepared and inoculated with the *R. solani* fungus and incubated for 5 days at an incubator temperature of 29-32°C. The sorghum seeds were transferred to a petri dish that *R. solani* had overgrown. The tested petri dishes were incubated in the dark for 4 days. After the incubation, the sorghum sprouts were transferred to a petri dish containing sterile filter paper moistened with sterile distilled water. Petri dishes were incubated in a dark storage room for 7 days until disease symptoms could be observed. During the incubation period, the moisture from the filter paper must be continuously maintained (Yulia and Widiyantini 2018; Kalymbetov et al. 2023).

**Simultaneous inoculation.** In this treatment, sorghum seeds and sclerotia of the *R. solani* fungus were inoculated simultaneously in a petri dish containing PDA media and then incubated in a dark place for 4 days. After the incubation period, the sorghum sprouts are transferred to a petri dish containing sterile filter paper, which has been moistened with sterile distilled water. Furthermore, the growth of the tested sorghum sprouts was observed in the same way as the separate treatment (1), which is observing the growth of the sprouts for 7 days in a dark room.

#### Observation parameters and data collection

The resistance level of sorghum was observed and analyzed based on germination percentage, Sprout Growth, Growth Tolerance Index (GTI), and Disease incidence. The germination percentage value is very important because it is an important step in evaluating the success of planting seeds.

**Germination percentage.** Criteria for seed germination is when the coleoptile and radicle have grown  $\pm 2$  mm long. If these two structures have grown to the specified length, the seeds classified have been germinated (Kasmiyati et al. 2015; Guragain et al. 2023). Seed germination observations were carried out immediately after the sorghum seeds and *R. solani* were incubated for 4 days or on the first day the sorghum sprouts were removed from the *R. solani* pathogen.

$$\% \text{ Germination} = \frac{\Sigma BK}{\Sigma TB} \times 100\%$$

Where:

$\Sigma BK$ : Number of seeds germinated

$\Sigma TB$ : Total number of seeds

**Sprout growth.** The growth of sorghum sprouts includes radicle length, plumule length, and sprout length, calculated on the 7<sup>th</sup> day. Each parameter measurement used a ruler and expressed in centimeters (cm).

**Growth Tolerance Index (GTI).** The Growth Tolerance Index (GTI) parameter was used as an assessment of the percentage growth of sorghum plants in sorghum seedlings without *R. solani* (control) and sorghum seedlings treated with *R. solani* (Akinci and Akinci 2010). The following formula was used to calculate (GTI):

$$GTI = \frac{1}{n} \sum_{i=1}^n \frac{PCri}{Pci} \times 100$$

Where:

N: Morphological parameters used (germination percentage, radicle length, and coleoptile length)

PCri: The total number of treated plant parameter values

Pci: Control plant parameter values

**Disease incidence.** The resistance of sorghum varieties was observed from planting sorghum sprouts until the appearance of the initial symptoms in the observed plants. The time interval for observation was from day 1 to day 7 after being inoculated into a petri dish containing sterile filter paper. The diseases observed were the incidence of reddish to gray blotches (blight) on stems, leaves, sheaths, and root rot of sorghum (Sukto et al. 2021). Disease incidence can be calculated using the formula:

$$DI = \frac{n}{N} \times 100\%$$

Where:

DI : Disease Incidence

n : Number of Diseased Plant In sample unit,

N : total Number of Plants in sample unit.

Based on the value of disease incidence (%), an average score of disease attacks is obtained. The average score of disease attack and resistance category ranged from 0-100%. Where 0-10%: Very strong resistant (VR); >10-20%: Strong resistant (SR); >20-40%: Moderate resistant (M); >40-60%: Weak resistant (WR); and > 60-100% Sensitive (S).

### Data analysis

The data obtained were analyzed statistically using a two-way ANOVA analysis of variance with the SAS program version 9.1.3, and the differences in values between treatments were further tested using Duncan's test at the level of  $\alpha = 5\%$ .

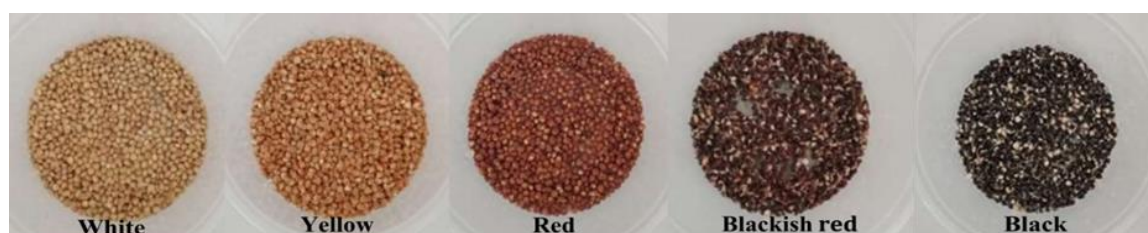
## RESULTS AND DISCUSSION

### Resistance test of local sorghum varieties (*Sorghum bicolor*)

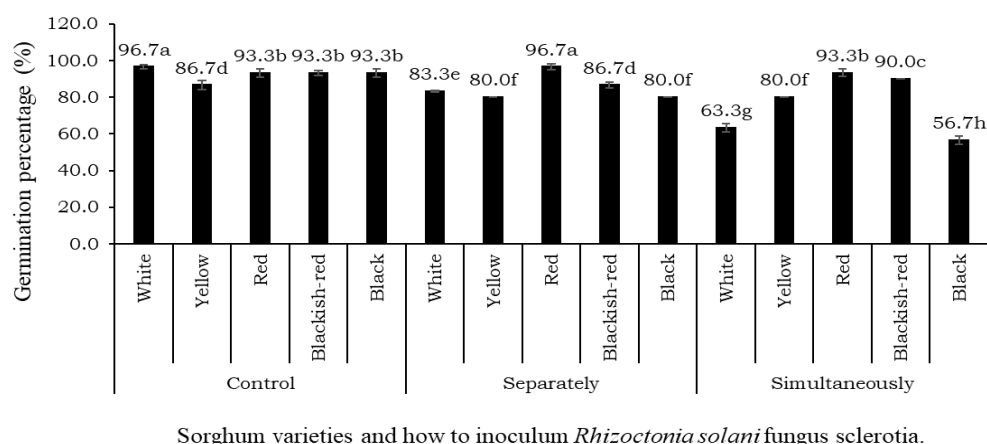
The tests for sorghum resistance to *R. solani* infection were carried out on five sorghum varieties with different seed coat colors: white, yellow, red, blackish-red, and black (Figure 1). The fungal inoculum was given through two methods: simultaneously and separately, and the five sorghum varieties showed different responses to *R. solani*. Variety treatment and the fungal inoculum method significantly influenced germination, sprout growth, growth tolerance index (GTI), and disease incidence (KP).

### Seed germination

Sorghum seeds from the five varieties tested showed different germination responses to *R. solani* fungal infection treatment (Figure 2). Based on Figure 2, the *R. solani* fungus inoculum treatment significantly inhibited the germination of the five sorghum varieties compared to the control treatment. In the control treatment's response, the percentage of sorghum seed germination was highest in the white variety (96.7%), significantly different from the yellow variety (86.7%). However, the difference between the red, blackish red, and black varieties was not significantly different because the three varieties have the same germination percentage, 93.3%. Meanwhile, in the separate *R. solani* treatment, the highest germination percentage was found in the red variety (96.7%), significantly different from the yellow and black varieties (80.0%). Apart from that, when the *R. solani* treatment was given simultaneously, the germination response of the tested sorghum was highest in the red variety, reaching 93.3%, which was significantly different from all other tested varieties. Based on the response to the *R. solani* treatment, red varieties tended to be more resistant when treated separately compared to simultaneously and in the control. This result indicates that local red sorghum varieties may have a better resistance to the *R. solani* fungus when *R. solani* treatment was given separately. Furthermore, when the *R. solani* treatment was given simultaneously, the local red sorghum variety outperformed other varieties with a higher germination percentage. This situation shows that this variety remains resistant even in more challenging conditions when the *R. solani* fungus is presented simultaneously in the growth medium.



**Figure 1.** Sorghum varieties tested for resistance to *R. solani* infection



Sorghum varieties and how to inoculum *Rhizoctonia solani* fungus sclerotia.

**Figure 2.** The percentage of the five sorghum varieties' germination in the control treatment, separated *R. solani* inoculation treatment, and simultaneous *R. solani* inoculation treatment on the germination medium. The average value in the treatment group followed by the same letter shows that there is no significant difference in the DMRT test (Duncan's Multiple Range Test) with a test level of  $\alpha = 0.05$

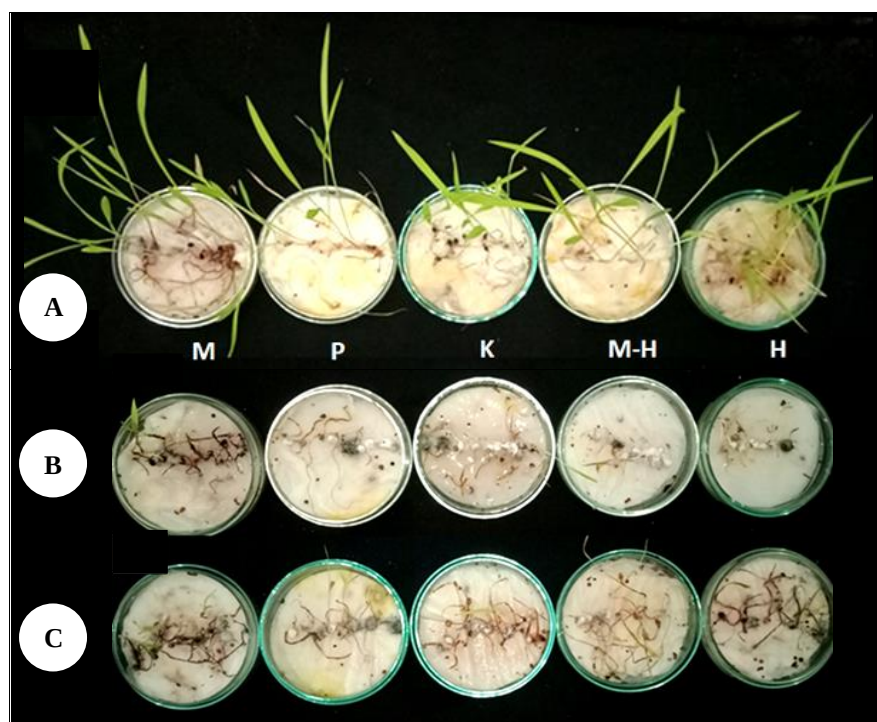
These results indicate that the local red sorghum variety is classified as more resistant to *R. solani* attack than other tested varieties. This difference in germination percentage response between varieties can be projected because each variety has different genetic resistance in responding to the *R. solani* pathogen. This condition follows the study of Mofokeng et al. (2017) and Ulhaq and Masnilah 2019 that a plant's resistance can be influenced by varietal factors (genes) and the plant's environment. Each variety has genetic diversity that can affect the response to *R. solani* pathogen. Therefore, in this research, the red variety is assumed to have genes more resistant to *R. solani* pathogenic fungus than other varieties. Based on a study by Dwipa et al. (2018), a plant is classified as resistant if the characteristics of the plant affect the level of damage caused by pathogen attack and usually have a higher silicate content than susceptible varieties. Apart from that, environmental factors in the form of germination medium can also be expected to influence the germination percentage value in the results of this study. The high germination percentage value in the red variety is probably due to the slow growth of *R. solani* fungal hyphae in covering sorghum seeds on the *R. solani* treated separately compared to simultaneously. Therefore, the chance of successful germination percentage for sorghum seeds to germinate is higher. *Rhizoctonia solani* sclerotia takes around three days or 72 hours to develop into young hyphae. Based on the study of Yang et al. (2022), Xue et al. (2018), and Abdoulaye et al. (2022), young hyphae of *R. solani* can produce the polygalacturonase enzyme able to attach and colonize plant seeds, causing rot when the seeds begin to germinate, or damping off. This effect shows that it is very likely that the fungal inoculum remains stored in infected seeds, causing rot and reducing the germination percentage. Istikorini and Sari (2020) reported that *R. solani* could cause the percentage of senger seeds that germinate and are healthy to be relatively low, where 64% of the seeds did not germinate, and 36% failed to germinate due to infection from the *R. solani* pathogen inoculum.

### Sprout growth

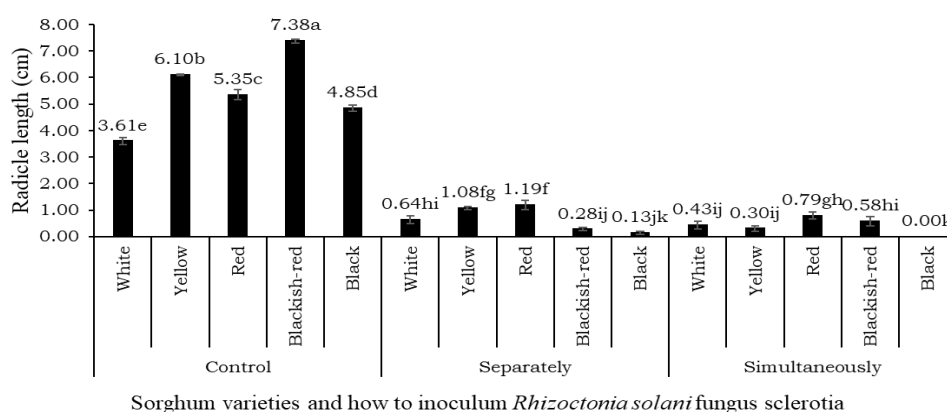
Visually, the five local sorghum varieties (white, yellow, red, blackish red, and black) treated with *R. solani* simultaneously and separately showed a significant reduction in growth ability compared to the ones in control (Figure 3). The different growth abilities of each variety against *R. solani* infection can be used to determine the most resistant variety.

Figure 3 shows that the five local sorghum varieties tested experienced susceptibility caused by significantly different *R. solani* fungus treatments compared to the controlled ones. The five varieties infected with the *R. solani* pathogen visually experienced root rot symptoms; the red variety still resisted this pathogen. Therefore, it can be observed visually that the variety with the highest resistance is the red variety. This variety is assumed to be resistant because it has a high growth capacity and the ability to adapt to *R. solani* stress better than other varieties. In this way, the variety still has the opportunity to grow even though it has been infected since the beginning of germination. The ability of *R. solani* infection to sorghum sprouts' growth can also be seen based on the growth parameters observation (radicle length, coleoptile length, and sprout length), which can be measured at the end of the observation.

Based on the observations in Figure 4 show that giving *R. solani* treatment, both simultaneously and separately, significantly inhibited the growth of radicle length in the five local sorghum varieties compared to the control. When treating *R. solani* separately, we can see the length of the radicle as follows: red variety (1.19 cm), followed by the yellow (1.08 cm), white (0.64 cm), blackish red variety (0.28 cm), and the lowest is the black variety (0.13 cm). The same patterns happened in the simultaneous treatment, where the highest radicle length was recorded in the red sorghum variety, only reaching 0.64 cm, significantly different from the black variety (0.00 cm).

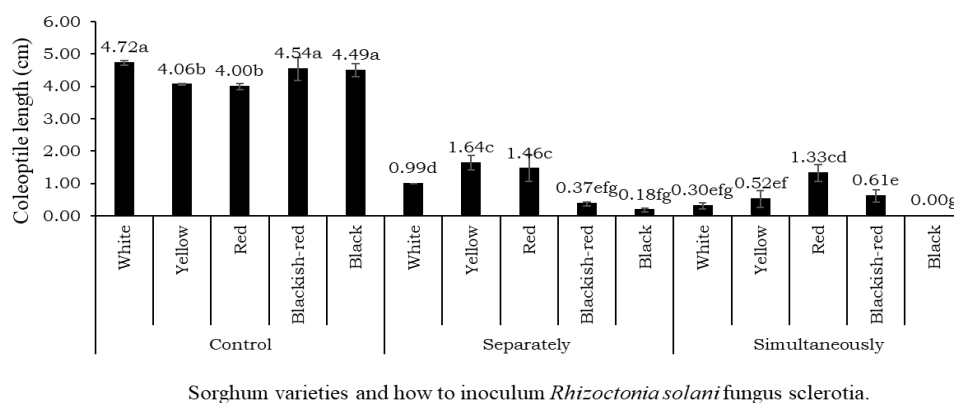


**Figure 3.** Sprout Growth of the five sorghum varieties in the control treatment (A), *R. solani* inoculation given separately (B), and simultaneously (C) on germination media. M: red variety, P: white variety, K: yellow variety, M-H: blackish-red variety, H: black variety



Sorghum varieties and how to inoculum *Rhizoctonia solani* fungus sclerotia

**Figure 4.** The radicle length of the five sorghum varieties in the control treatment, separated *R. solani* inoculation treatment, and simultaneous *R. solani* inoculation treatment on the germination medium at the end of the study. The average value in the treatment group followed by the same letter shows that there is no significant difference in the DMRT test (Duncan's Multiple Range Test) with a test level of  $\alpha = 0.05$



Sorghum varieties and how to inoculum *Rhizoctonia solani* fungus sclerotia.

**Figure 5.** The coleoptile length of the five sorghum varieties in the control treatment, separated *R. solani* inoculation treatment, and simultaneous *R. solani* inoculation treatment on the germination medium at the end of the study. The average value in the treatment group followed by the same letter shows that there is no significant difference in the DMRT test (Duncan's Multiple Range Test) with a test level of  $\alpha = 0.05$

This shows that *R. solani* can inhibit the growth of the radicle length of sorghum plants. Sorghum plant roots are one of the plant organs that are very sensitive to pathogen attack. This is because the roots are directly in contact with the growth medium, where the pathogenic fungus *R. solani* develops. Sorghum plant roots are important in absorbing water and nutrients and maintaining plant stability. Also, roots are a potential entry point for the fungus *R. solani*, which can cause root rot disease. If the roots of a sorghum plant are infected with root rot disease by *R. solani*, the root function will experience abnormal symptoms, causing stunted and even death. This follows Pinaria and Assa (2022) study, which states that plants with root systems damaged by pathogens can continue to grow but are stunted at various stages of growth. In addition, the study of Sturrock et al. (2015) said that the pathogen *R. solani* can cause root damage in wheat (*Triticum aestivum*) and oil seed rape (OSR, *Brassica napus*) plants, including reducing the number of primary roots, root volume, root surface area and breaking off of the tested plants' roots. This is also supported by the study of Da Silva et al. (2017) and Abdoulaye et al. (2022), which states that infection by *R. solani* inhibits and reduces root length and number of root tips in corn plants. The short length of sorghum radicles inoculated with *R. solani* shows that *R. solani* releases pathogenic enzymes that can break down the cell walls of plant roots and continue to spread to the coleoptile tissue of the sprouts, causing the sprouts to become slimy and die.

In addition, *R. solani* treatment given simultaneously and separately can also inhibit the growth of the coleoptile length of sorghum sprouts. Based on the growth in coleoptile length of the 5 tested sorghum varieties, there appeared to be a significant effect between the sorghum varieties in control and with *R. solani* treatments given separately or simultaneously (Figure 5).

When treating *R. solani* separately, the variety that experienced the highest growth in coleoptile length was the yellow variety (1.64 cm), significantly different from the red variety (0.20 cm). In addition, simultaneous *R. solani* treatment can also inhibit the growth of coleoptile length. It is known that the highest growth in coleoptile length in simultaneous *R. solani* treatment was found in the red variety, reaching (1.30 cm), which was significantly different from the black variety (0.00 cm). Thus, it can be concluded that *R. solani* treatment can also cause growth inhibition in the length of the tested sorghums' coleoptiles. This is caused by the ability of *R. solani* to infect young sorghum sprouts by attacking the sheath that protects the coleoptile. Attacks by *R. solani* in the coleoptile growth phase can disrupt the transport of water and nutrients that support the growth of sprouts, resulting in the growth of coleoptile length in sprouts being hampered.

The coleoptile is a leaf protector in a small hood that covers the true leaves before they appear. Like the radicle, the coleoptile is also a part of the plant in a developmental stage easily susceptible to disease. Root rot disease in sorghum plants caused by *R. solani* also causes damage to the coleoptiles. The characteristics of sorghum coleoptiles affected by root rot disease caused by *R. solani* are brownish-red to black necrosis; the infection continues to

develop to cause rot and can have fatal consequences in the death of the sprouts.

When a pathogen infects the coleoptile, the roots will likely be damaged and not function optimally in forming true leaves. This is following the study of Yellareddygar et al. (2014) and Dewi et al. (2020), disease infections in the stem and midrib affect the ability of the xylem and phloem in the process of transporting water, nutrients, and other nutrients for photosynthesis purposes, which can cause rice yield loss. Based on a study by Nafriana et al. (2013), *R. solani* attacked 8 out of 12 tested sorghum lines in the vegetative growth phase, including necrosis of the roots, midribs, stems, and sorghum leaves.

Figure 6 shows that the *R. solani* fungus inoculum significantly affects the growth of the tested sorghum plants' sprouts compared to the control treatment. In the control treatment, the highest sprout length was found in the red-black variety (11.9 cm), significantly different from the white variety (8.3 cm). Meanwhile, in the separately given *R. solani* treatment, the highest sprout length was found in the red variety, which was not significantly different from the yellow variety (2.7 cm), but the height of these two varieties was significantly different from the other tested varieties. In the simultaneous *R. solani* treatment, the variety with the highest sprout length was the red variety (2.1 cm), significantly different from the other test varieties.

The results of this study indicate that *R. solani* treatments given simultaneously and separately had a significant inhibitory impact on the tested sorghum sprouts, with varied responses based on the type of variety used. This is supported by a study (Yulianti et al. 2016) that the *R. solani* inoculation process carried out along with mixing organic materials before the incubation cause the tested cotton seeds not to germinate, or if they germinate, their growth will be stunted. *Rhizoctonia solani* treatment can cause low sorghum sprout length. This is because *R. solani* is a fungal pathogen that causes serious disease-damping in plants. *Rhizoctonia solani* infects all plant organs (seeds, roots, stems, and leaves); therefore, plants cannot grow and develop optimally. Based on the study by Bandte et al. (2013) and Lamichhane et al. (2017), *R. solani* is one of the pathogens that causes damping off, such as seed rot before germination or death of young plant seeds. Apart from that, based on research (Koch et al. 2022), seeds that come into direct contact with *R. solani* fungal inoculum cause high levels of damage to developing corn sprouts. The *R. solani* pathogen can attack the test sorghum seedlings in the pre-growth phase and when the seeds grow, causing seedling rot. *Rhizoctonia solani* infection in the pre-growing phase causes the radicle (root system) and coleoptiles to experience necrosis. In comparison, *R. solani* attack after sorghum growth causes root rot, stem fall, leaves turn yellow, wilt, and die.

### Growth Tolerance Index (GTI)

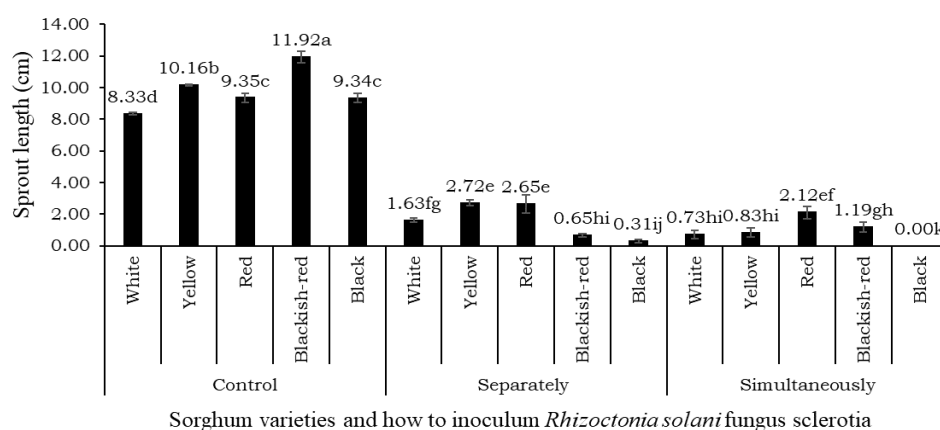
The Growth Tolerance Index (GTI) value predicts the stress level of the five local sorghum varieties treated with *R. solani* or without *R. solani* (control). The lower GTI value indicates a high level of susceptibility to the *R. solani*

pathogen. Conversely, the higher GTI value indicates a high resistance level of the variety to the *R. solani* pathogen. Based on Figure 7, the GTI value of sprouts of the five local sorghum varieties was negatively influenced by *R. solani* treatments given simultaneously or separately. The calculation of GTI values for sprout growth of 5 local sorghum varieties was done using observational parameter data of germination percentage, radicle length, and coleoptile length.

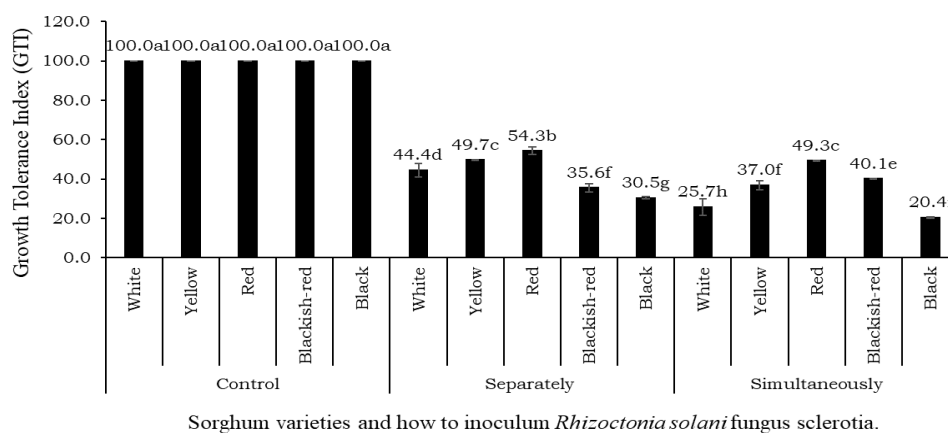
Figure 7 shows the observation results on the GTI values for the 5 sorghum varieties in the control treatment, which significantly differed from the 5 sorghum varieties given *R. solani* treatment separately or simultaneously. In the control treatment, the tested sorghum plants tended to have higher GTI values and did not differ significantly between the test varieties; this shows that the growth and development of the tested plants were better without the *R. solani* treatment. Meanwhile, when treating *R. solani* separately, it was observed that the red variety had the highest GTI value, reaching (54.3), significantly different from the black variety, which had the lowest GTI of (30.5).

This shows that the red variety has a higher resistance level to the effects of *R. solani* treatment given separately.

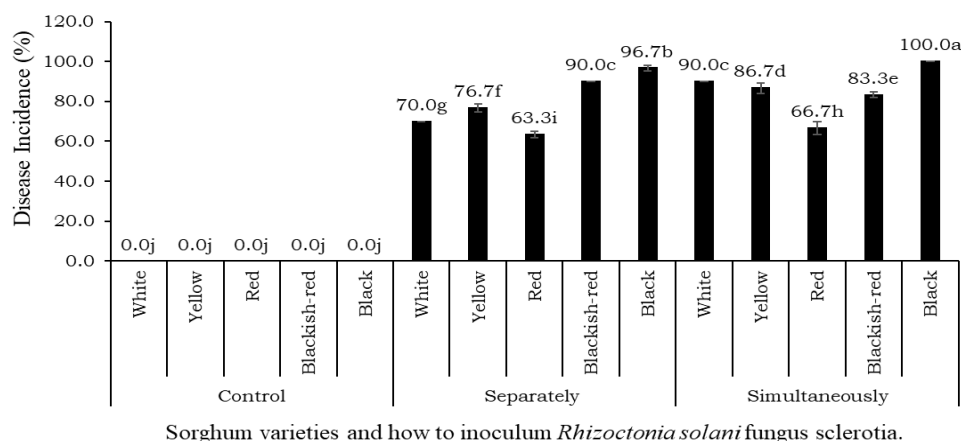
Similar patterns happened in the simultaneous treatment of *R. solani*, where the highest GTI value was found in the red variety (49.3), significantly different from the black variety (20.4). These results illustrate that local red sorghum variety has better resistance in overcoming the negative impacts of *R. solani*, both in separate and simultaneous treatments. This follows the study of Wiszniewska et al. (2017), which states that high and low GTI values in tested plants can be caused by the treatment factors given. Apart from that, the results of the study by Çikili et al. (2020) also support the previous statement that the GTI value in the Sunflower test plants was greatly influenced by the Cadmium treatment given by showing inhibition of the growth of the roots and shoots of the test plants. Therefore, in this research, the GTI values of the 5 tested sorghum varieties provide important information for farmers. This information indicates that among the 5 tested varieties, the red variety has the highest resistance level to the *R. solani* pathogen.



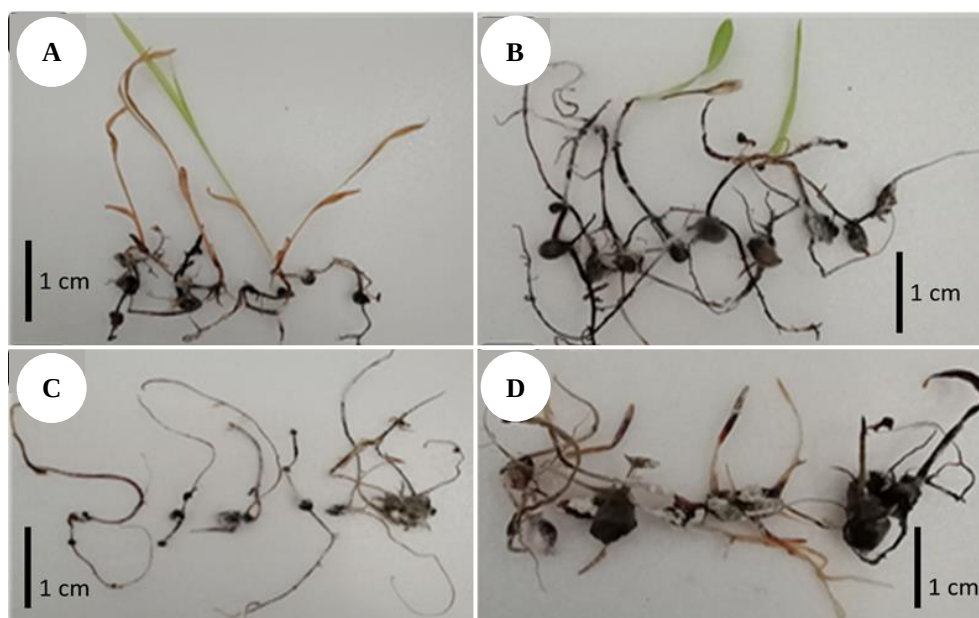
**Figure 6.** Sprout length of five sorghum varieties in the control treatment, separated *R. solani* inoculation treatment, and simultaneous *R. solani* inoculation treatment on the germination medium at the end of the study. The average value in the treatment group followed by the same letter shows that there is no significant difference in the DMRT test (Duncan's Multiple Range Test) with a test level of  $\alpha = 0.05$



**Figure 7.** Growth Tolerance Index (GTI) of the five sorghum varieties in the control treatment, separated *R. solani* inoculation treatment, and simultaneous *R. solani* inoculation treatment on the germination medium at the end of the study. The average value in the treatment group followed by the same letter shows that there is no significant difference in the DMRT test (Duncan's Multiple Range Test) with a test level of  $\alpha = 0.05$



**Figure 8.** The percentage of disease incidence of the five sorghum varieties in the control treatment and *R. solani* fungus inoculation was given separately and simultaneously on germination media at the end of the study. The average value in the treatment group followed by the same letter shows that there is no significant difference in the DMRT test (Duncan's Multiple Range Test) with a test level of  $\alpha = 0.05$



**Figure 9.** The morphology of red variety sorghum sprouts treated with *R. solani* inoculum separately (A) and simultaneously (B), and black variety separately (C) and simultaneously (D) at the end of the study

### Disease incidence

Disease incidence was used as a calculated observation parameter to determine the resistance level of local sorghum varieties to the *R. solani* pathogen that causes root rot. The results shown in Figure 8 clearly show that *R. solani* treatment, whether given simultaneously or separately, significantly impacted the disease incidence levels of the five local sorghum varieties tested. High and low disease incidence values can indicate the level of susceptibility or resistance of a test variety to the pathogen.

Based on Figure 8, when treating *R. solani* separately, the red variety showed the lowest disease incidence rate (63.3%) compared to other varieties. It significantly differed from the black variety, with a disease incidence

rate reaching 96.7%. Likewise, in the *R. solani* treatment given simultaneously, the red variety still showed the lowest disease incidence (66.7%) compared to the black variety (100%). In this study, although all tested sorghum varieties were categorized as susceptible to *R. solani* based on the disease incidence rate, the red variety stood out as the variety with the highest resistance to the *R. solani* pathogen with the lowest disease incidence rate (63.3%).

The low value of disease incidence in the local red variety shows that this variety has a much better defense mechanism or is resistant to the *R. solani* pathogen compared to other tested varieties. This finding is strengthened by the research results by Rusae et al. (2018), who said that the incidence and severity of stem rot disease

caused by *R. solani* was significantly lower in local white and red sorghum varieties than in other tested varieties. On the other hand, black sorghum varieties show the highest disease incidence values or are more susceptible to the *R. solani* pathogen. This may be due to the lack of a strong defense mechanism against the *R. solani* pathogen in these varieties. Moreover, using local sorghum varieties resistant to root rot disease caused by *R. solani* is an effective, economical, and environmentally friendly measure. The ability of local plants to withstand the pathogen causing the disease is also reported by Dinanty et al. (2022), out of the 55 tested sorghum genotypes, five of them, namely genotypes 24 (IS 185510), 33 (Demak 5), 17 (ICSV 93036), 31 (Demak 2), 43 (Local Bima 3), have been identified as local sorghum varieties that are resistant to the leaf rust disease and also have a high grain weight per panicle. Therefore, the results of the disease incidence values in this study can inform farmers that the variety with the highest level of resistance to the *R. solani* pathogen is the red variety, and the black variety is the most susceptible. Thus, this has important implications in selecting local sorghum varieties more resistant to the *R. solani* pathogen in agricultural practices. This will positively impact increasing sorghum crop yields in the local area. Meanwhile, for susceptible varieties (black varieties), more intensive protective measures are needed so that these varieties can be protected from the *R. solani* pathogen that causes root rot.

The results of morphological observations showed that the red variety was the most resistant (Figure 9 a and b); even though it was infected with the *R. solani* fungus, there was still growth, significantly different from the black variety (Figure 9 c and d). The categorization of red varieties as resistant varieties can be seen from the results of variance analysis tests (percentage of germination, sprout growth, GTI value, and disease incidence). The highest resistance value was found in the red variety with *R. solani* treatment given separately, with a germination percentage value of 96.7%, radicle length (1.2 cm), coleoptile length (1.5 cm), sprout length (2, 7cm), GTI value (54.3) and incidence value 63.3%. Based on the average disease incidence score, the highest resistance value is found in the red variety, with a score of 63.3%, significantly different from the black variety whose disease incidence score is 96.7%, categorized as very susceptible (SR). From the susceptibility test results, it can be concluded that the red variety is the most resistant variety. Plant resistance to pathogens can be seen from the plant's ability to limit the growth of disease-causing pathogens. Generally, a plant is resistant to a pathogen if it can grow and develop better than similar test plants that receive the same pathogen treatment. Therefore, the results of this study can be concluded as follows: There are differences in resistance responses between 5 local sorghum varieties to *R. solani* infection, which were applied simultaneously and separately. Among the five sorghum varieties tested, the red variety had the highest resistance with germination percentage (96.7%), radicle length (1.2 cm), coleoptile length (1.5 cm), sprout length (2.7 cm), GTI value (54.3), and disease incidence value (63.3%). However, to find out

more about the resistance mechanisms of local red sorghum varieties to the *R. solani* pathogen, further research needs to be done at the molecular and genetic level. With a deeper understanding of the genetic aspects of red sorghum resistance to these pathogens, we can develop more complex gene-based breeding strategies to effectively increase the resistance of sorghum plants. This will make a significant contribution to disease management in sorghum crops and increase overall agricultural productivity.

## ACKNOWLEDGEMENTS

The author would like to thank Ellon Belwan Cornelius Mau, for his assistance in providing the seeds of five local sorghum varieties of East Nusa Tenggara (NTT). In addition, the author also thanks Ms. Nanuk Tri Setyorini, for assistance and facilities in the form of tools and materials needed during the research.

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