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Diversity of leaf rust resistance in Indonesian sorghum genetic resources

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Abstract. *Dinanty F, Wirnas D, Trikoesoemaningtyas, Nawangsih AA. 2022. Diversity of leaf rust resistance in Indonesian sorghum genetic resources. Biodiversitas 23: 2570-2579.* Sorghum is an alternative source as a substitute for rice. Leaf rust (*Puccinia purpurea*) is a constraint in sorghum cultivation and resistance to rust disease is a condition for releasing sorghum varieties. This study aimed to screen sorghum accessions for resistance to leaf rust disease. The screening was carried out on 48 sorghum accessions and seven check varieties, namely Numbu, Kawali, Bioguma 1, Pahat, Soraya 1, Soraya 2, and Soraya 3. Evaluation of leaf rust resistance under field conditions was carried out in IPB University experimental field Cikarawang-Bogor (275 asl) from February 2021 to June 2021. The experiment was conducted in an augmented design with four replications for check varieties. Identification of leaf rust resistance was also carried out using molecular markers designed from the Lr34 and Rp1D genes. The results revealed that 5 genotypes, namely genotypes 31 (Demak 2), 24 (IS 18551), 43 (Lokal bima 3), 17 (ICSV 93036), and 33 (Demak 5) were resistant to leaf rust with high yield potential. The results of identification using molecular markers showed that all accessions had Lr34 and Rp1D gene fragments.

Keywords: Feed sources, food crops, genetic variation, leaf rust, *Puccinia purpurea*, sorghum

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a cereal crop belonging to the Poaceae family with the fifth-highest production in the world after wheat, rice, corn, and barley (FAO 2021). Sorghum has wide adaptability due to which it can grow and develop well on marginal lands (Rao and Kumar 2013). Sorghum is used as food and fodder in many provinces of Indonesia. According to Sulistyawati et al. (2019), the local varieties have high protein, medium fat, and carbohydrates, which it is widely cultivated as a source of healthy food. At present, sorghum cultivation has expanded in various regions, but there is no official report from the Ministry of Agriculture regarding the planted area and national sorghum production. An increase in planting intensity is usually followed by an increase in pest and disease attacks. Global climate changes, such as rising temperatures and rainfall, also support the development, spread, and changing patterns of pest and disease attacks (Rao and Kumar 2013; Doody 2020). Sorghum disease is also caused by fungi, viruses, bacteria, and nematodes, as is common in other crops (Das and Rajendrakumar 2016; Prom 2017). One of the diseases of sorghum is leaf rust caused by *Puccinia purpurea* (White et al. 2012). Symptoms of leaf rust are indicated by the presence of reddish or brownish-colored spots on the leaf surface (Thakur et al. 2007). Leaf rust infects sorghum from the flowering phase to the seed filling phase (White et al. 2014; CABI 2019). Yield reduction due to leaf rust was reported as 13.1% in hybrid sorghum (White et al. 2012). In Indonesia, studies on rust disease in sorghum are still limited to testing resistance of lines or prospective varieties (Novemprirenta et al. 2013; Andriani 2019; Rifka et al. 2020). Soenartiningsih et al. (2013) reported several sorghum diseases including leaf rust in Central Sulawesi. Rusae and Metboki (2018) also reported resistance of local varieties to sorghum leaf rust in East Nusa Tenggara.

Resistance to rust disease has become a requirement for the release of sorghum varieties listed in the regulation of the Minister of Agriculture of the Republic of Indonesia Number 38 in 2019. This encourages the development of sorghum varieties that not only improve yield potential and quality, but also improve resistance to leaf rust disease. Until now, the varieties of sorghum that have been released are only moderately resistant to leaf rust, such as Numbu, Kawali, Bioguma 1, and Pahat (Balitbangtan 2013).

Disease races develop continuously so that varieties or genotypes that are reported to be resistant can experience decreased resistance (Neik et al. 2017). This allows the development of resistant varieties with new sources of resistance. For this reason, it is necessary to identify genotypes from a wider genetic background as a source of resistant traits (Bhaskar et al. 2020). Identification of genotype resistance to rust disease can be done through field screening using sensitive plants as spreader row (SR) for inoculum sources. Several studies have succeeded in identifying disease resistance using the SR method, including the identification of leaf rust, powdery mildew, net blotch, and spot blotch on barley (Hickey et al. 2017),

wheat (Mu et al. 2019), and sorghum leaf rust (Rifka et al. 2020).

Identification of potential resistant parents can also be carried out using molecular markers to identify the presence of resistance genes. The use of molecular markers helps identify genes that facilitate the marking of disease resistance genes, thereby enabling selection based on molecular markers that are not influenced by the environment (Das and Rajendrakumar 2016). The resistance genes to leaf rust have been identified in sorghum, including Rp1D and Lr34. The genes play a role in encoding the ATP-binding cassette (ABC) which also provide resistance to leaf rust disease in wheat (McIntyre et al. 2004; Krattinger et al. 2013; Schnippenkoetter et al. 2017). Schnippenkoetter et al. (2017) also carried out the transformation of Lr34 gene into sorghum plants and results showed that plants showed partial resistance and inhibited the development of sporulation or spore formation until harvest. In this study, screening was carried out for resistance to leaf rust disease in 48 local and introduced sorghum accessions as well as several national varieties based on the intensity of disease incidence and identification of resistance using molecular markers linked to the Rp1D and Lr34 genes. Accessions or varieties that are classified as resistant can be used as parents in generating breeding material to produce sorghum varieties that are resistant to leaf rust disease.

MATERIALS AND METHODS

Study area and genetic material

Fifty-five genotypes consisting of 48 accessions from the gene bank collection of ICABRIOGRAD, 4 national varieties, and 3 genotypes from IPB University were used in this study. The experiment was carried out between February and June 2021 at the Cikarawang Experimental Field of IPB University (275 asl), Bogor, Indonesia and the Molecular Biology Laboratory, Indonesian Center for Biotechnology and Agricultural Genetic Resources Research and Development (ICABIOGRAD). The climatic conditions during the growing season are shown in Table 1.

Table 1. Climatic conditions during growing season of February-June 2021

	February	March	April	May	June
T (°C)	25.5	25.9	26.7	26.8	25.8
Tmax (°C)	33.4	32.5	32.2	32.5	31.7
Tmin (°C)	24.4	22.4	23.4	23.0	22.1
RF (mm)	24.4	4.4	28.1	16.5	12.4
RH (%)	81.0	87.0	84.2	84.2	86.2
SI (Cal/cm^2)	468.0	141.4	530.6	488.0	383.1
WR (m/s)	3.1	2.9	3.8	2.9	2.6

Note: T: Average temperature, Tmax: Temperature maximal, Tmin: Temperature minimum, RF: Rainfall, SI: Solar radiation intensity, RH: Relative humidity, WR: Wind rapidity. *Meteorology, Climatology and Geophysical Agency (BMKG) of Bogor, Indonesia (February 2021-June 2021)

Field screening for resistance of sorghum genetic resources

The study was initiated by planting 2 susceptible genotypes as spreader rows, namely Super 1 and Super 2 which were naturally infected around the experimental area and between replicates as a source of leaf rust inoculation. The spreader row was planted in two rows between the replicates and one row around the experimental field. The tested genotypes were planted 28 days after planting the SR genotypes. The experiment was conducted in an augmented design with four replications for the check varieties. Each replication consisted of 12 different test genotypes and seven checks so that a total of 19 experimental units in one replication. Sources of diversity in the analysis of variance came from genotypes (genotype collection of ICABRIOGRAD) and check varieties, namely Numbu, Kawali, Bioguma 1, Pahat, Soraya 1, Soraya 2, and Soraya 3. The experimental plot was measured 1.5 m × 3 m and the seeds were planted with a spacing of 75 cm × 15 cm. Urea, SP36, and KCl fertilizers were applied at a dose of 150, 100, 100 kg/ha, respectively. Urea fertilizer was given twice, i.e. 2/3 parts at planting and 1/3 parts when the plants were four weeks after planting (WAP), while SP-36 and KCl were given at the time of planting.

Observations were made on 10 sample plants per experimental unit for grain weight per panicle (g). Leaf rust resistance was investigated based on the green intensity or the amount of chlorophyll, number of stomata, incubation period, and severity of leaf rust disease. The incidence of rust disease on leaves was measured using a score of 1 to 6 modified Cobb's (Thakur et al. 2007), where; 0: No visible infection; 1: 1-5% infected leaf area; 2: 6-10% infected leaf area; 3: 11-25% infected leaf area; 4: 26-40% infected leaf area; 5: 41-64% infected leaf area; 6: 65-100% infected leaf area. Leaf rust scoring was carried out when plants were 70 DAP, 80 DAP, and 90 DAP, scoring data were converted to the area under disease progress curves (AUDPC) based on Simko and Piepho (2012) using formula (1) and disease severity based on Djafaruddin (2008) with use formula (2).

The severity of disease was used as a reference in determining the level of resistance to leaf rust disease in accordance with the procedures for releasing varieties in Indonesia as follows: Very resistant (HR): DS<5%; Resistant (R): 5%< DS >20%; Moderate resistance (MR): 20%<DS > 40%; Susceptible (S): 40%<DS>60% and Very Susceptible (HS): DS>60.

Microscopic observation of the fungus (*Puccinia purpurea*) was carried out using a light microscope. Leaf rust spores were taken using tweezers, then placed on the surface of a glass slide and 10 µL of distilled water was added. Observations were made using a magnification of 40X. Identification was carried out at the Plant Clinic, Department of Plant Protection, IPB University. The value of variance component was obtained from the separation of the expected mean squares using SAS 9.4 and the broad-sense heritability was calculated based on formula (3). Correlation analysis was carried out using software R 4.1.10 with the package "Agricole" and "Pheatmap" used to analyze the correlation between heatmap and heatmap cluster. These two programs can be accessed via

https://cran.r-project.org/web/packages.

$$AUDPC = \sum_{i=I}^{n-1} \frac{y_i + y_{i+1}}{2} \; x \; t_{i+1} - t_i \quad [1]$$

$$DS = \frac{\sum(n_i \times v_i)}{n \times z} \; x \; 100\% \quad [2]$$

$$h^2_{bs} = \frac{\sigma^2 g}{\sigma^2 p} \quad [3]$$

Where: yi+l= i-th observation data + 1; yi= 1st observation data; ti+1= i+1 observation time; ti: Time of the first observation; n: Total observations; DS: Severity of disease; ni: Number of plants on the I score; vi: I score value; n: Number of plants observed; Z: Highest score.

Identification of sorghum germplasm resistance to disease based on specific primers

DNA extraction and amplification

DNA extraction was carried out by a modified CTAB method based on Doyle and Doyle (1987). 0.5-1.5 g of young leaves were harvested at 2 WAP. The young leaves were ground in a mortar to which 800 L of 2% CTAB extraction buffer + 2% PVP was added with the addition of 3 L of 2-mercaptoethanol 2%. A DNA sample was dissolved in 100 L of 1% TE + RNAse buffer.

Amplification by PCR was carried out in a 10 µL total reaction volume containing 2 µL of 20 ng/µL DNA template, 1 µL forward and reverse primers with final concentration 0.5 µM each, 5 µL of 2X MyTaqTM HS Red Mix PCR Kit (Bioline, USA), and 2 µL of ddH_2O. The PCR program carried out was pre-denaturation at 95°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing for 30 seconds, extension at 72°C for 45 and final extension at 72°C for 7 minutes.

Visualization of PCR products

Amplification results were visualized using 2% agarose gel (0.6 g agarose with 30 mL TBE buffer 0.5x) and then electrophoresed at 100 volts for 30 minutes. After completion, the gel was immersed in 1% EtBr (*Ethidium bromide*) solution followed by documentation using Gel Doc EZTM (Bio Rad, USA).

Confirmation of the genes

The presence of Rp1D gene was confirmed according to McIntyre et al. (2004) and the primers used for Lr34 genes were designed using multiple alignments based on information from Schnippenkoetter et al. (2017) and NCBI (Table 2).

RESULTS AND DISCUSSION

Identification of the fungus (*Puccinia purpurea)* as a leaf rust pathogen

Leaf rust infects sorghum plants at about 60 DAP when the plant has begun to enter the flowering phase. In general, leaf rust attacks occur when the sorghum plant enters the flowering and seed filling phase (CABI 2019). Infection was characterized by red or brownish spots on the leaf surface which will then develop into pustules. The result of microscopic observation revealed that leaf rust of sorghum was caused by *Puccinia purpurea* (Figure 1).

Symptom of leaf rust is reddish spots that develop to form pustules and contain uridospores (Thakur et al. 2007; Ramirez-Cabral et al. 2017). Tsukiboshi (2002) reported that *Puccinia purpurea* belongs to the order Pucciniales which has 3 stages of development, namely uredo, telia, and basidial. Urediospores produced in uredinia are elliptical or ovoid and serve as a source of inoculum or a source of infection in leaves (Burhanuddin 2015; Zhao et al. 2016).

Yield performance and germplasm resistance of sorghum in the field using natural inoculation

In this study, a spreader row was used which was left naturally infected as a source of inoculum for screening the severity of leaf rust disease in sorghum plants. This was done because *Puccinia purpurea* which causes leaf rust is an obligate biotrophic fungus that cannot reproduce without living plants as hosts. Leaf rust reproduces as haustoria formed in the host tissue to obtain nutrients and suppress the host's defense response to infection (Lorrain et al. 2019).

The average performance of grain weight per plant, number of stomata, leaf greenish intensity, day the plant was infected, disease severity of leaf rust at the age of 70, 80, and 90 of the tested genotypes are shown in Table 3, while the performance of each genotype is shown in Table 4. The results of t-test showed that there was no difference in the average grain weight per panicle and the number of stomata, while the leaf greenish intensity was significantly different between the accessions and check lines. The accessions became infected more quickly than the check lines, but the severity of disease in the accessions was generally lower than the check lines (Table 3). This has raised the hope of finding a source of germplasm that is resistant to leaf rust disease.

Table 2. List of primers used in the identification of sorghum resistance to leaf rust disease

Gene	Primer sequence (5'-3')	Annealing temperature (°C)	Amplicon size (bp)
Rp1D	F: CCATCGTCACACGCGAGAGATT R: GGTATGTTCTAGCTTCATGCGCAAT	55.7	400
Lr34 Like 1	F: GGTGACCGAACAGGACATCT R: AGTCGACCGAGCAGGATCTA	59.0	229
Lr34 Like 2	F: CCTAAGAGTGGGGTGTTCCA R: TCACCCTCGAATTTCTTTGC	59.0	245

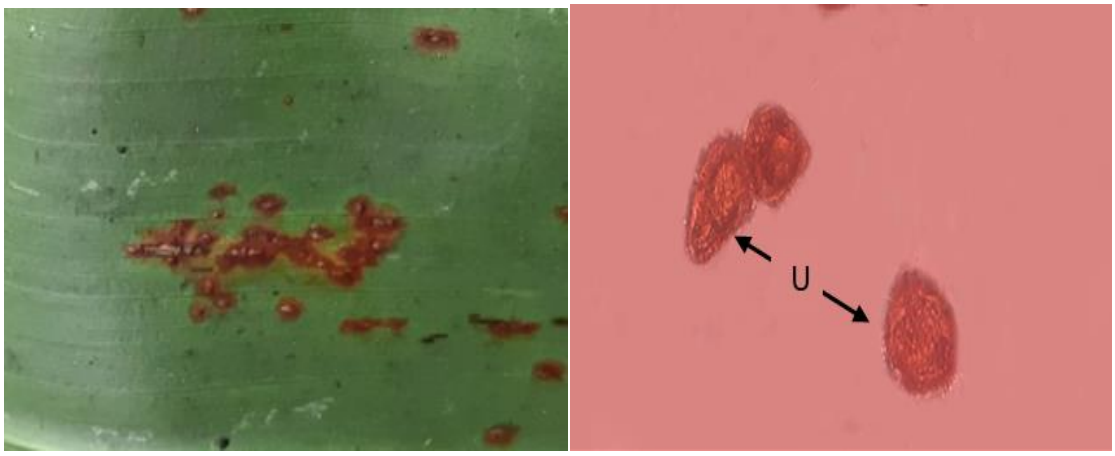

Figure 1. Microscopic identification. A. Rust pustules on leaves, B. Shape of urediniospore of *Puccinia purpurea*

Table 3. The performance of sorghum genetic resources

Characters	Accessions (48 genotypes)	Check lines (7 lines)	All tested genotypes (55 genotypes)	Pr(>|t|)
Grain weight per panicle (g)	36.3	35	35.8	0.4
Number of stomata	23.2	21.3	22.5	0.1
Leaf greenish intensity (CCI Units)	52.05	46.1	49.9	0.0
Days to infection (DAP)	41	59	48	0.0
Disease severity at 70 DAP (%)	1.6	2.9	2.1	0.7
Disease severity at 80 DAP (%)	3.49	6.7	4.7	0.7
Disease severity at 90 DAP (%)	3.76	7.4	5.1	0.2

It was also observed that there were 28 accessions that had grain weights per panicle better than the average of the best check varieties in conditions infected by natural rust disease inoculum. The check genotypes with the highest weight grain per panicle were Numbu and Soraya 2. There were also 28 accessions that had a greater number of stomata than the check varieties. Stomata act as sites of gas exchange in the process of photosynthesis, but stomata are also a pathway for fungal invasion. Damage to stomata leads to cell death, an attempt by plants to prevent fungal infection (Ye et al. 2020).

Leaf greenish intensity was measured using SPAD to indicate the amount of chlorophyll present in the leaves. The greenish leaf intensity values in the tested sorghum genotypes ranged from 37.2 to 84 CCI units. The amount of chlorophyll was found to be different in healthy and infected plants. Plants infected with the pathogen experience a decrease in the amount of chlorophyll followed by stomatal conductance and a decrease in the rate of photosynthesis (Gortari et al. 2018). The incubation period for leaf rust on sorghum tested in this study ranged from 62 to 82 DAP (Table 4). Genotypes 34 and 35 were recorded as the genotypes with the fastest incubation period, i.e. 62 DAP and genotype 25 had the longest incubation period, i.e. 82 DAP. Nine genotypes tested had an incubation period of 76 DAP and three genotypes (33, 18, 47) had an incubation period of 80 DAP. According to Draz et al. (2015) genotypes with a long incubation period can be classified into partial resistance in the form of slow rusting.

The result showed that 41 genotypes were highly resistant to leaf rust with a severity value of <5%, 5 genotypes showed leaf rust resistance with a disease severity value ranging from 5.7% to 10.9%, one moderate genotype showed 39.5% disease severity value and one susceptible genotype exhibited a disease severity value of 40.8%. The identification of natural rust resistance of sorghum leaves was also carried out by Sharma et al. (2012) who identified 105 genotypes of sorghum that were resistant to leaf rust using natural inoculation. The lines identified as resistant to leaf rust allowed them to become resistant parents or controls (Sserumaga et al. 2020).

The area under the disease progress curve (AUDPC) graph was used to determine the development of leaf rust disease over a certain period. AUDPC graphs were created using disease severity values (i.e. rust disease severity at 70 DAP, 80 DAP, and 90 DAP for each genotype. The AUDPC graph (Figure 2) shows the curve area of each tested sorghum genotype, where genotype 35 and genotype 45 have the largest curve area among other genotypes. This is also in line with the severity of the disease (Table 4).

According to Forbes et al. (2014), the same or similar AUDPC levels can cause by long-term initial infection from the initiating disease that attacks the genotype and slow or rapid disease progression. The more severe the illness, the larger the area of AUDPC and vice versa. Since there was no significant difference between the AUDPC value and the severity of the disease, similar curved areas occur. The AUDPC should be carried out regularly so that it can indicate disease incidence, be able to analyze

genotype responses and predict potential yield losses in the field (Gangwar 2013; Sserumaga et al. 2020).

Differences in disease severity may be influenced by genetic factors that lead to differences in disease responses such as hypersensitivity responses, production of reactive oxygen species (ROS), cell wall modification, stomatal closure or the production of proteins or compounds that inhibit disease progression (Panchal and Melotto 2017; Andersen et al. 2018). In addition, leaf rust can also trigger the emergence of other infectious diseases such as stem rot, anthracnose and seed rot (Sharma et al. 2012).

Most of the genotypes tested in this study were classified as resistant to leaf rust disease. This was presumably because the SR method could not provide adequate inoculum to infect plants. Another weakness of the SR method is that it is difficult to avoid an attack on other diseases that cause differences in disease severity (White et al. 2015). To ensure the availability of sufficient inoculum can be tested with semi-artificial inoculation or testing in a greenhouse in the seedling phase (Thakur et al. 2007). Sharma et al. (2012) identified leaf rust by field screening and greenhouse screening and the results showed that the genotype resistance to leaf rust in the field was reduced by 10% when tested in a greenhouse. Mengistu et al. (2020) conducted a leaf rust resistance test in the field for two seasons to determine the consistency of sorghum resistance. Rifka et al. (2020) conducted a leaf rust resistance test and found a significant effect of genotype and environmental (GxE) interactions to sorghum resistance.

Correlation among characters

There was a negative, but weak correlation between disease severity at 70, 80, and 90 DAP and seed weight per panicle. This indicates that leaf rust disease almost did not affect the yield (Figure 2). Similar results have been reported by Rifka et al. (2020) where no correlation was found between the severity of rust disease and severity (70 DAP to 90 DAP) with seed weight per plant in sorghum lines tested using natural inoculums. This is because leaf rust attacks around 60 DAP, where it is suspected that the plant has completed the maximum vegetative phase and photosynthate has accumulated in the stem (parenchyma tissue) which can be used for basic metabolism, growth, or seed filling (Slewinski 2012; Bihmidine et al. 2015; Casto et al. 2018). There was a negative correlation between the number of stomata and disease severity at 70, 80, 90 DAP (Figure 2). This shows that the fewer the number of stomata, the lower the disease severity. Stomata are structures found in leaves and are a means of entry of pathogens into plants. Rust fungi penetrate leaves at the opening of stomata, then develop substomata vesicles, hyphae, and haustorial hyphae that form in host mesophyll cells (Lorrain et al. 2019; Ye et al. 2020).

The leaf greenish intensity or amount of chlorophyll with disease severity at 70, 80, 90 DAP was negatively and weakly correlated (Figure 1). This is presumably because the amount of chlorophyll that was damaged due to disease did not differ between genotypes, but the level of damage was more determined by the amount of chlorophyll possessed by a genotype. Greener leaves have higher amounts of chlorophyll and chlorophyll also plays a role in providing the first resistance and/or contributing to the production of ROS through photosynthetic metabolism (Kretschmer et al. 2020; Yahya et al. 2020). For that Yahya et al. (2020) explained the importance of considering the timing of data collection for chlorophyll content in order to obtain values relevant to rust infection or spore spread. Therefore, measurement was performed using SPAD when sorghum entered the age of 60 DAP and entered the flowering phase. The incubation period was calculated using the time of day after planting and appearance of visible rust infection on the plant. The Incubation period was strongly positively correlated with disease severity at 80 and 90 DAP and weakly positively correlated with crop seed weight (Figure 2).

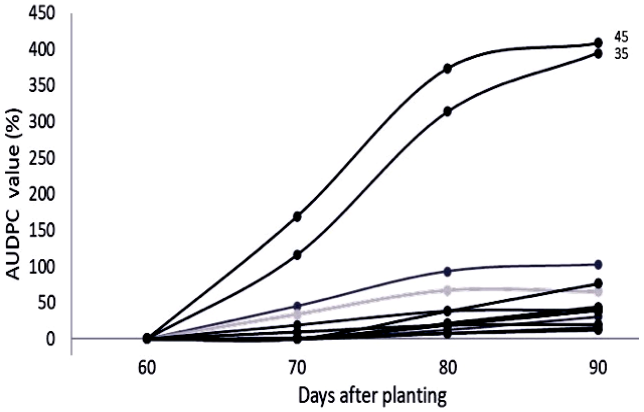

Figure 2. The AUDPC graph of plant infected by leaf rust disease

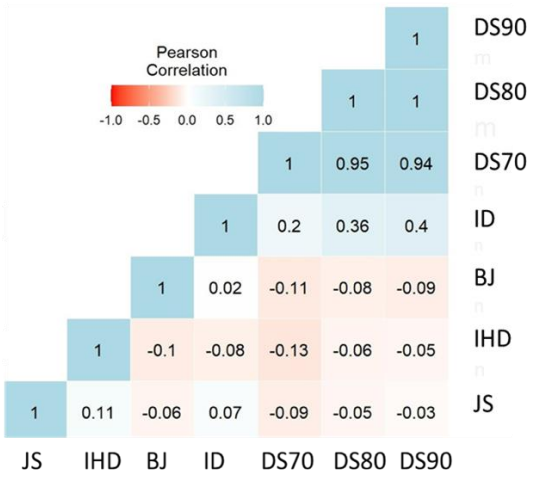

Figure 3. Correlation among characters using heatmap. BJ: Grain weight per panicle (g), JS: Number of stomata, IHD: Leaf greenish intensity (CCI/Chlorophyll Content Index Units), DS: Disease severity (%) at 70, 80, and 90 days after planting

Table 4. Grain weight per panicle and parameters of sorghum resistance to leaf rust disease

Cd	BJ	JS	IHD	ID	Disease severity (%) (DAP)			Cl	Cd	BJ	JS	IHD	ID	Disease severity (%) (DAP)			Cl
					70	80	90							70	80	90	
1	41.6	19.4	51	70	0.3	1.3	1.3	HR	**29**	31.3	21.1	61.7	74	0	3.2	3.4	HR
2	38.8	25.4	52.8	0	0	0	0	HR	**30**	28.2	10.1	37.2	0	0	0	0	HR
3	30.8	24.4	55.6	0	0	0	0	HR	**31**	59	20.4	53.6	0	0	0	0	HR
4	34.3	19.6	50.7	0	0	0	0	HR	**32**	38	24.1	44.9	0	0	0	0	HR
5	35.5	20.1	51.2	0	0	0	0	HR	**33**	51.4	14.1	44.1	80	0	3.5	3.7	HR
6	28.5	22.5	46.5	0	0	0	0	HR	**34**	29.9	20.1	44.8	62	6.8	6.6	6.4	R
7	29.2	23.8	47.8	0	0	0	0	HR	**35**	40.6	21.1	47.9	62	23.2	39.5	39.5	MR
8	39.6	19.8	49.3	74	0	0.9	1.4	HR	**36**	37.9	19.5	57.6	0	0	0	0	HR
9	21.8	19.4	53.5	0	0	0	0	HR	**37**	41.9	18.1	44.6	78	0	3.9	3.9	HR
10	27.1	29.1	51.5	72	0	3.4	3.8	HR	**38**	24.6	18.4	84	76	0	4.3	4.5	HR
11	26.3	26.1	50.9	76	0	5.7	5.7	R	**39**	28.8	31.5	51	76	1.9	1.9	2.1	HR
12	18.9	18.5	55.2	0	0	0	0	HR	**40**	35.3	18.8	37.9	78	0	7.7	7.7	R
13	30.5	25.1	53.7	0	0	0	0	HR	**41**	20.4	25.1	54.3	0	0	0	0	HR
14	16.2	36.5	54.4	0	0	0	0	HR	**42**	33.7	17.8	47.6	76	3.8	3.8	3.9	HR
15	25.8	28	55.3	0	0	0	0	HR	**43**	53.5	17.1	57.1	0	0	0	0	HR
16	24.2	36	61.4	0	0	0	0	HR	**44**	29.4	19	57.3	76	0	3.9	3.9	HR
17	53.3	25.7	58.7	76	0	3.2	4.3	HR	**45**	26.7	22	40.7	66	33.9	40.8	40.8	S
18	31.8	24.1	53.8	80	0	2.6	3.6	HR	**46**	38.3	26.1	56.2	76	0	3.2	4.6	HR
19	27.3	17.4	54	74	0	3.9	4.2	HR	**47**	45.4	25	55.8	80	0	1.5	1.7	HR
20	37.2	36	44.1	74	0	5.3	6.3	R	**48**	48	30.4	53.5	78	0	3.2	3.9	HR
21	21.3	28.1	53.4	0	0	0	0	HR	**Check**								
22	14.2	23.7	55	76	0	2.7	3.2	HR	**P1**	33.4	15	45	68	1.6	3.4	3.7	HR
23	30.2	23.7	51.6	74	0	1.9	3.5	HR	**P2**	47.8	15.6	50.3	76	0	1.4	2.3	HR
24	56.6	22.8	53.5	0	0	0	0	HR	**P3**	37.9	23	40.5	70	1.2	2.4	3.5	HR
25	35	22.1	51.2	82	0	0	2.1	HR	**P4**	38.4	27.8	51.2	66	3.6	10.6	12.2	R
26	38.1	25	52.5	0	0	0	0	HR	**P5**	38	24.2	42.3	62	6	10.2	10.3	R
27	50.1	26.1	43.3	0	0	0	0	HR	**P6**	42.5	25.7	47	60	5.8	13.8	13.9	R
28	16.3	25.8	44.5	76	9.1	9.6	10.9	R	**P7**	36.4	17.8	46.7	72	2	5.5	6.3	R

Note: Cd: Line code, BJ: Grain weight per panicle (g), JS: Number of stomata, IHD: Leaf greenish intensity (CCI/ Chlorophyll Content Index Units), Disease severity (%) DAP: Days after planting

The incubation period may be influenced by the virulence of a pathogen, where the pathogen develops pathogenic mechanisms followed by disease development. If the symptoms are widespread then the plant is susceptible, but if the symptoms are localized, it indicates that the resistance is active and can overcome the pathogen attack so that the plant is resistant (Andersen et al. 2018). If the plant can inhibit the development of the pathogen, then the disease has no effect on the plant during seed filling. Draz et al. (2015) stated that there is partial resistance in which the incubation period for leaf rust in infecting sorghum was long. In this study, there was one genotype that had the longest (82 DAP) incubation period, while 3 genotypes had an incubation period of 80 DAP (Table 3).

Heatmap cluster analysis (Figure 4) uses the "Euclidean" index for grouping homogeneous genotypes. The data can be visualized using color gradation with certain value intervals (Wilkinson and Friendly 2009). The color gradation used was red-blue, where the red color classified the genotypes as very similar or positively correlated (strong) and blue color classified the genotypes that have low or negative correlations with these characters. The heatmap of cluster showed that genotype 35 and genotype 45 were in one cluster, both of which have high disease severity scores (Table 3), both of which were also quite strongly correlated (yellow-reddish) with incubation period and not correlated with grain weight per panicle. The genotypes that had a correlation with yield were genotypes 24, 8, 37, and 43 with grain weights per panicle ranging from 39.6-56.6 g, and the four genotypes were categorized as highly resistant to leaf rust with disease severity of 0.0-3.9%.

Estimation of heritability values

The values of variance components and heritability in broad-sense are shown in Table 5. The heritability values ranged from 0.52 to 0.70. All observed characters had high heritability, except for disease severity at 70 DAP. High heritability value for leaf rust disease severity at 80 DAP and 90 DAP indicates that genetic variance is likely to be high between the assessed genotypes. The high heritability value indicates the large genetic influence of each plant and small environmental variation on this character. Ogunniyan and Olakojo (2014), and Figlan et al. (2018) also reported

high heritability values for disease severity characters. The high heritability value allows selection for improvement of leaf rust resistance in sorghum to be carried out in early generations. Estimation of heritability of leaf rust resistance characters has been reported in several previous studies, such as leaf rust on wheat (Figlan et al. 2018) and sorghum (Rifka et al. 2020), anthracnose in sorghum (Mengistu et al. 2020), and leaf rust in maize (Sserumaga et al. 2020).

Table 5. Heritability values of yield and resistance to rust disease of sorghum

Characters	σ^2e	σ^2g	σ^2p	h^2_{bs}
Grain yield per panicle	10.50	19.80	30.30	0.70
Number of stomata	2.50	6.30	8.80	0.70
Leaf greenish intensity	3.60	11.20	14.80	0.80
Incubation period	1.09	1.16	2.25	0.52
Leaf rust disease severity 70 DAP	0.01	0.00	0.01	0.10
Leaf rust disease severity 70 DAP	0.01	0.01	0.02	0.55
Leaf rust disease severity 70 DAP	0.01	0.01	0.02	0.52

Note: σ^2e: Environment variance, σ^2g: Genotypic variance, σ^2p: Phenotypic variance; h^2_{bs}: Heritability in broad-sense

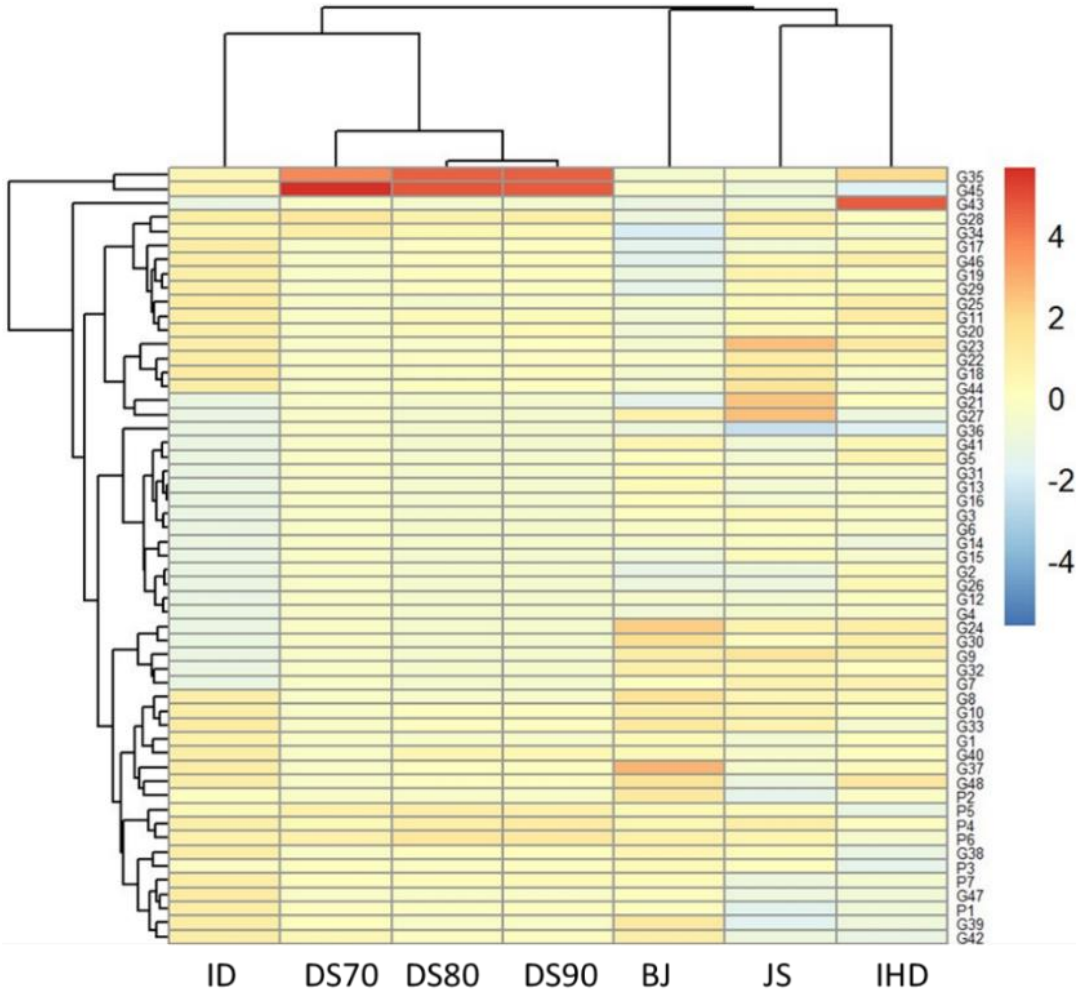

Figure 4. Heatmap cluster between resistance characters and grain weight per panicle. BJ: Grain weight per panicle (g), JS: Number of stomata, IHD: Leaf greenish intensity (CCI/Chlorophyll Content Index Units), DS: Disease severity (%) at 70, 80, and 90 days after planting

Identification of sorghum germplasm resistance to leaf rust disease based on specific primers

The disease resistance system in plants is very complex involving the presence of gene-for-genes, pathogen detection, signal transduction, and defense responses. The results of visualization of PCR products from this study using 3 sets of primers are shown in Figure 5. Identification of the Rp1D gene (Figure 5A) showed that all test genotypes had bands or DNA was successfully amplified with a band size of 400 bp. McIntyre et al. (2004) reported that there are 5 sets of sequence classes in identifying the Rp1D gene at the LG E sorghum locus which are located very close to each other**.** Identification of Lr34 Like 1 (Figure 5B) and Lr34 Like 2 (Figure 5C) showed that all genotypes had a band of 229 bp and 245 bp in size, respectively.

The results showed that all tested genotypes have the same bands and further confirmation was needed to see whether there were differences in sequences between genotypes. According to Krattinger et al. (2013), sorghum has two critical codons that are 'TTC' and 'TAT' which are related to disease susceptibility. The results of identification by molecular markers and by screening results in the field showed similar results, in which all genotypes were resistant to leaf rust, although in the field genotype 35 (Butter Biara) was moderate and genotype 45 (Sorghum pulut) was susceptible to leaf rust. This difference can occur because each genotype has differences in responding and activating resistance to disease attacks. From the results of this study, it was found that there was a diversity of resistance responses of 55 sorghum genotypes to leaf rust, with five genotypes, namely genotypes 31 (Demak 2), 24 (IS 18551), 43 (Lokal bima 3), 17 (ICSV 93036), and 33 (Demak 5) were resistant to leaf rust also had high grain weight per panicle. The results of molecular identification showed that all accessions had Lr34 and Rp1D gene fragments. The broad-sense heritability in this study ranged from 0.1 to 0.8. The correlation between disease severity and seed weight per panicle was negative and weak so that the severity of leaf rust disease was not followed by a decrease in yield.

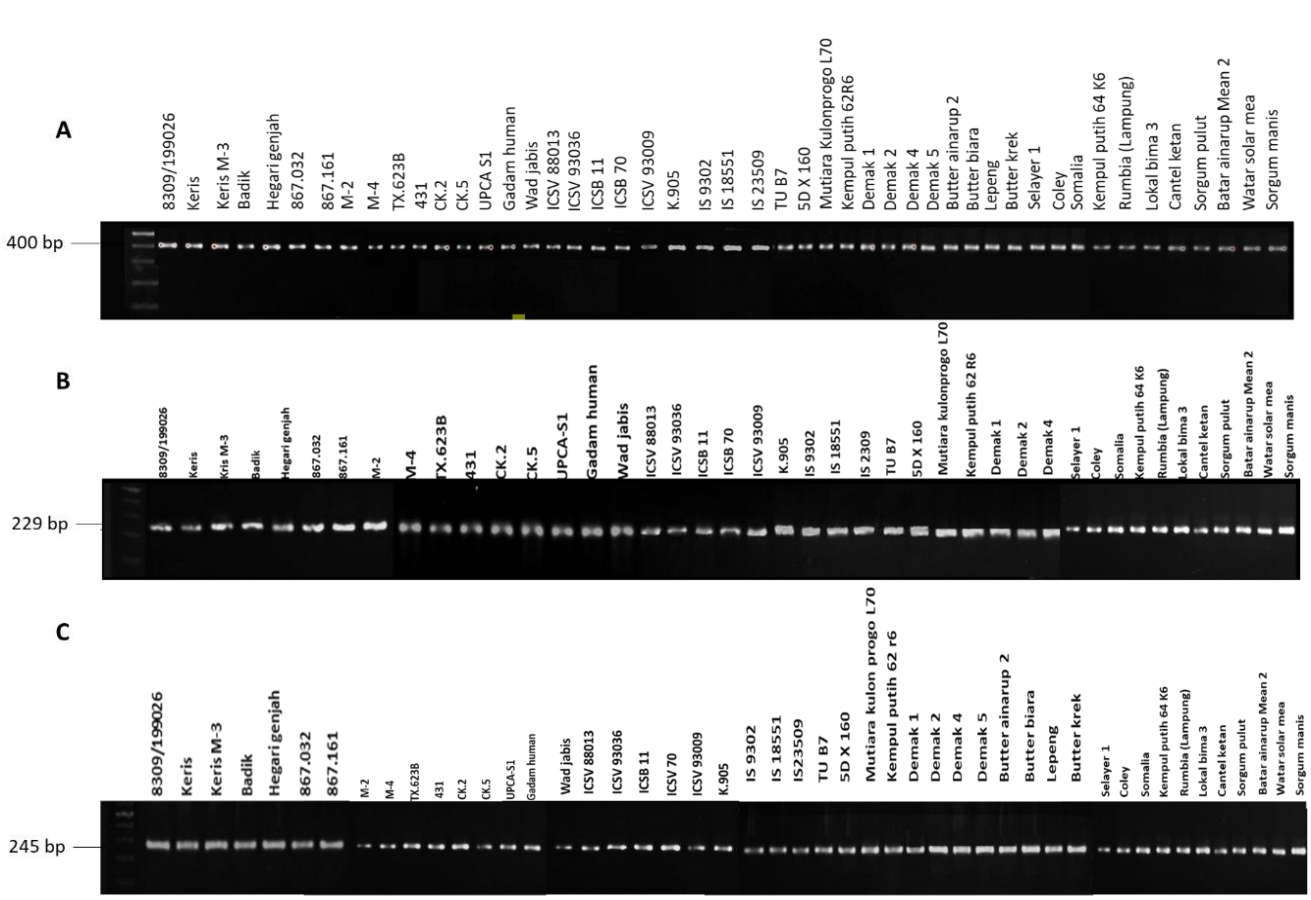

Figure 5. Visuals of PCR results using specific primer linked to leaf rust resistance genes in sorghum. Primer A: RpD1 gene, Primer B: Lr34 Like 1 gene, Primer C: Lr34 Like 2 gene

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