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GENOTYPE BY ENVIRONMENT INTERACTION AFFECTING LEAF RUST RESISTANCE IN SORGHUM

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SUMMARY

Screening for leaf rust resistance is vital in sorghum breeding programs. However, information on leaf rust disease severity and resistance in sorghum is still limited in Indonesia. This study reports sorghum leaf rust disease severity through genotype by environment (G × E) interaction. The genetic materials used in this study consisted of 13 sorghum advanced breeding lines generated from a cross between two national varieties, namely, UPCA-S1 and Numbu. Leaf rust disease severity was observed during August 2018 to December 2018 under artificial inoculation conditions at the Indonesian Cereals Research Institute (ICERI), Maros, South Sulawesi, Indonesia, and under natural infection during November 2018 to February 2019 at IPB University, Bogor, West Java, Indonesia. Disease severity was visually estimated using a standard scoring system from ICERI. The visual assessments of infection were made at 60, 70, and 80 days after sowing. Scores for each plot were recorded from 10 plants at each assessment time. Agronomic traits and yield were observed only in Bogor. The scores were converted into percentages for analysis of variance and categorized by the following procedures for variety release in Indonesia. Results showed that G × E interaction had a significant effect on leaf rust disease severity. The heritability of leaf rust disease severity was high for all phase assessments. The leaf rust disease severity of the sorghum lines varied depending on the growing location. Each location was suspected of having a different and more than one pathotype of rust pathogen. Numbu and Kawali were the most resistant and stable across locations. Three lines, namely, N/UP-139-5, N/UP-48-2, and N/UP-89-3, were classified as moderately resistant in Bogor but were classified as highly susceptible in Maros. Only line N/UP-166-6 was resistant to leaf rust disease in Bogor. Correlation analysis showed that leaf rust disease severity was not correlated with yield. Damage to leaves by the pathogen infection of leaf rust in the generative phase did not affect the filling of sorghum seeds. Given that photosynthate had accumulated in plant stems since the vegetative phase, the decrease in the amount of photosynthate during the generative phase due to pathogenic infections in leaf tissue had no effect on sorghum seed filling.

Keywords: Disease severity, hybridization, infection, pure lines, *Sorghum bicolor* L.

Key findings: The heritability of leaf rust disease severity was high for all phase assessments. The infection of leaf rust in sorghum was shown to have no correlation with yield in sorghum lines. Although the disease severity of leaf rust was very high, it was not followed by a decrease in yield even in susceptible lines.

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INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) become popular as an alternative source of food in Indonesia. The consumption of sorghum as a food source is allocated as part of a healthy life because sorghum has unique nutritional content (Salim *et al*., 2017). Sorghum breeding programs have produced 18 superior varieties that were released during 1973–2014. Most of the varieties were introduced from the International Crops Research Institute for the Semi-Arid Tropics and several countries, such as India, Philippines, Thailand, and China, that have passed yield and adaptation tests in Indonesia. Trikoesoemaningtyas *et al.* (2017) reported that efforts have been made to increase the availability of superior sorghum varieties that are more adapted to Indonesian agroecology through hybridization and then selection in target environments.

In addition to low productivity, disease infection is also a constraint in sorghum cultivation. One of the main diseases in sorghum is leaf rust caused by *Puccinia purpurea* (Bushnell and Roefls, 1985). Rust can cause a decrease in production by reducing the photosynthetic potential of the host plant and by diverting host photosynthesis results into their biomass, thus reducing carbon sequestration and causing more than 40% loss after infection (Dawson *et al*., 2005).

Rust disease control can be done in various ways, such as growing resistant varieties and applying fungicides. Disease control by growing resistant varieties is preferred by farmers, inexpensive, and environmentally friendly. Planting resistant varieties is intended to reduce disease incidence and yield loss. Thakur *et al.* (2007) reported that the rapid development of rust disease in an optimal environment could influence panicle exertion, decreasing production and quality of sorghum grain. White *et al.* (2015) reported that leaf rust could reduce yields by more than 13%.

Since 2018, resistance to leaf rust disease has become one of the requirements for the release of sorghum varieties in Indonesia. A sorghum variety can be released if it is resistant to leaf rust disease; however, the development of leaf rust-resistant sorghum varieties in Indonesia has only recently become a priority. The identification of resistant genotypes can be done through field screening by utilizing susceptible genotypes as inoculum donors. Direct screening in the field is an effective method because it represents the sorghum-growing environment. Mu *et al*. (2019) has screened rust resistance in 60 accessions of wheat and has succeeded in identifying 50 stripe-rust resistant accessions through field screening conducted through spreader row (SR) plant inoculation and natural infection. Differences in resistance among sorghum genotypes to leaf rust pathogens have been reported by Sharma *et al*. (2012) on 242 sorghum accessions in India and by White *et al*. (2015) on 10 sorghum genotypes in Australia.

Several studies have shown that resistance to rust is influenced by genotype × environment (G × E) interactions, such as in *Euthamia graminifolia* (Price *et al.,* 2004) and wheat (Rosewarne *et al.,* 2008; Mboup *et al.,* 2012; Yang *et al.,* 2013; Lohithaswa *et al.,* 2014). It indicates that the presence of G × E interactions in sorghum resistance to leaf rust cannot be ignored because it can cause errors in giving recommendations for varieties. Significant G × E interaction results in different phenotypes of accessions tested in different environments (Sillero *et al.,* 2017). Given that resistance to leaf rust is one of the indicators for the release of sorghum varieties in Indonesia, a study on G × E interactions is important. Knowledge on G × E interactions can assist breeders in identifying superior genotypes that are resistant to leaf rust disease, which can be recommended to farmers. This study reports on the effect of G × E interactions on leaf rust resistance in sorghum in two different environments and the correlation of leaf rust disease severity with yield.

MATERIALS AND METHODS

Agronomic traits and yield performance of sorghum lines

The study was conducted during November 2018–February 2019 at IPB University, Bogor Regency, West Java, Indonesia. The genetic materials used in this study were 13 sorghum advanced lines (N/UP-4-3, N/UP-17-10, N/UP-48-2, N/UP-82-3, N/UP-89-3, N/UP-118-3, N/UP-118-7, N/UP-124-7, N/UP-139-1, N/UP-139-5, N/UP-156-8, N/UP-159-9, and N/UP-166-6) from a cross between Numbu and UPCA-S1 developed by the Laboratory of Plant Breeding and Genetics, Departement of Agronomy and Horticulture, IPB University, Indonesia. These lines were developed through modified bulk method and will be released. However, information on their resistance to leaf rust disease is still needed. Two national varieties (Numbu and Kawali) were recommended as resistant varieties.

The experiment was conducted with a randomized complete block design (RCBD) with three replications. The experimental unit was a 3 m × 3.75 m plot with a plant spacing of 75 cm × 15 cm. Fertilizers consisting of urea, SP36, and KCl were applied at the rates of 100, 150, and 100 kg ha^{-1}, respectively. Two thirds of urea was applied at sowing with SP-36 and KCl. The rest of urea was applied at 7 weeks after sowing. Plot maintenance and pest control were conducted in accordance with standard practices for raising an ideal crop.

Observations were conducted on plant height (cm), stem width (mm), panicle length (cm), panicle weight (g), grain weight per panicle (g), 1000-grain weight (g), days to flowering (DAS), days to maturity (DAS), grain-filling period (days), and productivity (tons ha^{-1}). Observations were made on 10 randomly selected plants of each line in each replication. Obtained data were analyzed using SAS version 9.0 for variance analysis at α = 5%, continued with LSD test best on fix model.

Resistance to leaf rust in sorghum lines

The screening for leaf rust resistance was conducted in two locations. The first experiment was conducted during Aug.—Dec. 2018 under artificial inoculation at Indonesian Cereals Research Institute (ICERI), Maros Regency, South Sulawesi, Indonesia. The average values of maximum temperature, minimum temperature, humidity, and rainfall were 32 °C, 24 °C, 76%, and 220 mm, respectively. The second experiment was conducted during Nov. 2018–Feb. 2019 under natural infection at IPB University, Bogor Regency, West Java, Indonesia. The average values of maximum temperature, minimum temperature, humidity, and rainfall were 32 °C, 23 °C, 85%, and 329 mm, respectively (Table 1). In each location, the experiment was conducted with RCBD with three replications.

The first experiment used the susceptible varieties Super 1 and Super 2 as inoculum donors by planting these two rows around the trial site and between replications as SRs. Urediniospores were collected from the infected leaves. These leaves were washed with aquadest and air-dried. Leaf bases were soaked using sucrose for approximately 3 h and then were stored in plastic bags containing wet towels to remove excess moisture. After 48 h, the harvested urediniospores were placed in a glass beaker and then suspended in water. Spore concentration was adjusted by using a hemocytometer. The SR plants were inoculated with a suspension of urediniospores (approximately 6×10^4 urediniospores ml^{-1} water) approximately 3 weeks after sowing. Inoculation was carried out in the afternoon under sufficiently moist soil conditions. The trial plants were planted after the SR plants showed leaf rust symptoms. Each genotype was grown in a plot with three rows 5 m in length with 75 cm between rows and 20 cm between plants.

Table 1. Climate data of the locations of leaf rust screening.

Parameters	Maros[a]	Bogor[b]
Minimum temperature (°C)	24	23
Maximum temperature (°C)	32	32
Average temperature (°C)	28	26
Average humidity (%)	76	85
Average rainfall (mm)	220	329
Rainy days (days)	13	22

[a]Meteorology, Climatology and Geophysical Agency (BMKG) of Maros, Indonesia (Aug.–Dec. 2019).
[b]Meteorology, Climatology and Geophysical Agency (BMKG) of Bogor, Indonesia (Nov. 2018–Feb. 2019).

In the second experiment, the sorghum genotypes were naturally infected. Each genotype was planted in five rows with a spacing of 75 cm between rows and 15 cm between plants. Urea, SP-36, and KCl fertilizers were applied at the rates of 150, 100, and 100 kg ha^{-1}, respectively. In the first experiment, urea was applied at the rate of 300 kg ha^{-1}. The number of lines planted was 10. The climatic conditions at the location for the first experiment (Maros) and the second experiment (Bogor) are presented in Table 1.

Disease severity was visually estimated using a standard scoring system from the ICERI (unpublished) (Table 2). The visual assessments of infection were made at 60, 70, and 80 DAS. Scores for each plot were recorded form 10 plants at each assessment. The scores were accumulated into the formula of disease severity described by Suriani *et al*. (2018):

$$DS = \frac{\sum n_i v_i}{NZ} \times 100\%,$$

where:

DS = Disease severity
n_i = Number of plants from each of infection score
v_i = Value of infection score
N = Number of recorded plants
Z = The highest value of score

The DS values were used as a reference in determining the level of resistance to leaf rust disease through the following procedure for variety release in Indonesia:

Highly resistant (HR): DS $\leq$ 5%
Resistant (R): DS > 5%–20%
Moderately resistant (MR): DS > 20%–40%
Susceptible (S): DS > 40%–60%
Highly susceptible (HS): DS > 60%

Bartlett's M test for homogenity was done to detect any significant differences among genotypes. The obtained data were analyzed using SAS version 9.0 for the analysis of variance at α = 5% based on a random model with genotype and environment as random variables. If the F test was significant, the DMRT test was performed. Correlation analysis was used to determine the relationship between yield and leaf rust disease severity by using R 3.5.1 software. The variance partition of expected mean squares (Table 5) was performed to estimate broad-sense heritability.

RESULTS

Genetic variability in sorghum lines

The analysis of variance showed that genotypes have a significant effect on all agronomic traits (Table 3). The value of the coefficient of variation (C.V.) was relatively small with a range of 1.32%–13.49%.

The sorghum lines had heights ranging from 154.75 cm to 256.58 cm, which can be categorized into medium height (151–225 cm) and tall (226–300 cm) (Elangovan *et al*. 2014). The highest plant height was shown by Numbu with an average of 256.58 cm, whereas the shortest was shown by Kawali with an average of 154.75 cm. These lines were expected to have shorter plant heights or were not significantly different from Kawali. All of the sorghum lines, except for N/UP-89-3, were significantly different

Table 2. Score for leaf rust disease reactions.

Score	Description
0	No symptoms
1	Spots covering about ≤ 5% leaf areas
2	Spots covering about ≤ 10% leaf areas
3	Spots covering about ≤ 25% leaf areas
4	Spots covering about ≤ 50% leaf areas
5	Spots covering about ≤ 75% leaf areas
6	Spots covering about ≤ 90% leaf areas
7	Spots covering about ≤ 95% leaf areas
8	Symptoms more severe, covering more than 95% leaf areas

Source: ICERI (unpublished).

Table 3. Analysis of variance of the agronomic traits of sorghum lines.

Traits	Mean Squares		Coefficient of variation (%)
	Replications	Genotypes	
Plant height	265.05	2901.40**	6.72
Stem width	5.57	4.30*	7.63
Panicle lenght	0.50	17.42**	4.91
Panicle weight	587.10*	485.28**	12.71
Grain weight per panicle	310.15*	258.18**	13.13
1000-grain weight	3.34	25.78**	3.87
Days to flowering	7.02	19.31**	2.16
Days to harvesting	7.47*	14.86**	1.32
Grain-filling period	15.02**	8.88**	4.86
Productivity	1.08*	0.92**	13.49

**. * = Significant at 1% and 5% probability, respectively.

Table 4. Variation in agronomic trait measurements at sorghum lines in Bogor.

Genotype	PH	SW	PL	PW	GW	TGW	DF	DM	GF	Y
N/UP-4-3	221.39[b]	18.57	18.30	98.18[b]	71.21	31.88[b]	69.00[b]	102.00[b]	33.00[ab]	3.93[b]
N/UP-118-3	247.53[b]	21.05[ab]	18.22	92.95	66.56	26.84	73.00[b]	103.33[b]	30.33	3.95[b]
N/UP-118-7	225.32[b]	19.37	17.68	94.01	65.66	26.54	73.00[b]	102.67[b]	29.67	3.59[b]
N/UP-124-7	223.87[b]	17.21	16.96	76.65	56.88	31.08[b]	69.67[b]	102.67[b]	33.00[ab]	3.18
N/UP-139-1	243.57[b]	17.63	16.50	84.72	62.19	35.05[b]	72.33[b]	102.00[b]	29.67	3.41
N/UP-139-5	180.31[b]	17.18	16.29	58.10	42.27	28.02	74.33[b]	105.33[ab]	31.00	2.22
N/UP-156-8	231.66[b]	17.65	18.68	83.34	61.64	30.83[b]	73.00[b]	103.67[b]	30.67	3.30
N/UP-159-9	234.78[b]	16.60	20.54	91.25	74.51[b]	29.44	70.00[b]	102.00[b]	32.00[a]	3.68[b]
N/UP-166-6	187.77[b]	17.96	19.80	70.64	53.42	26.99	69.33[b]	102.67[b]	33.33[ab]	3.23
N/UP-17-10	254.62[b]	19.91	18.31	91.58	62.15	27.61	73.00[b]	102.00[b]	29.00	3.53[b]
N/UP-48-2	225.73[b]	17.47	19.42	79.98	59.04	32.61[b]	73.00[b]	102.67[b]	29.67	3.30
N/UP-82-3	219.56[b]	19.05	18.19	87.55	63.47	27.25	73.67[b]	103.33[b]	29.67	3.72[b]
N/UP-89-3	170.84	17.38	15.41	59.00	44.78	29.44	69.67[b]	103.67[b]	34.00[ab]	2.36
Numbu	256.58	17.90	19.06	97.91	73.45	35.54	73.33	102.00	28.67	4.08
Kawali	154.75	17.66	25.76	77.67	58.02	28.07	65.00	95.00	30.00	2.71
$LSD_{0.05}$	24.57	2.32	1.53	17.63	13.40	1.93	2.58	2.26	2.51	0.75

a = Significantly different from Numbu at LSD 5%; b = significantly different from Kawali at LSD 5%; PH = plant height (cm); SW = stem width (mm); PL =panicle length (cm); PW = panicle weight (g); GW = grain weight per panicle (g);TGW = 1000-grain weigth (g); DF = days to flowering (DAS); DM = days to maturity (DAS); GF = grain filling period (days); Y = productivity (tons ha^{-1}).

in height from Kawali (Table 4), but was not significantly different from Numbu, which is one of the parents of the tested lines.

The stem width of the sorghum lines ranged from 16.60 cm to 21.05 mm. Most of the sorghum lines have small stem widths (<20 mm) (Elangovan *et al*. 2014). However, line N/UP-118-3 had a larger stem width than Numbu and Kawali (Table 4). Sorghum with medium-to-tall stems with small stem widths are prone to lodging, especially when planted during the rainy season. Therefore, it is important to improve lodging resistance by selecting for stronger stems with larger widths. The average panicle length of the sorghum lines ranged from 15.41 cm to 25.76 cm, which are classified as short (11–20 cm) and moderate (20–30 cm) categories (Elangovan *et al*. 2014). The national variety, Kawali, had the longest panicle length of 25.76 cm, and line N/UP-89-3 had the shortest panicle length of 16.14 cm. All sorghum lines tested had panicle lengths that were not significantly different from Numbu and Kawali (Table 4).

In addition to plant height, panicle weight is one of the selection characters used to select these lines during their early generations. These advanced sorghum lines had panicle weights and grain weight per panicles ranging from 59.00 g to 98.18 g and 42.27 g to 74.51 g, respectively. The sorghum line with panicle weight greater than Kawali was N/UP-4-3 (98.18 g), and the sorghum line with grain weight panicle greater than Kawali was N/UP-159-9 (74.51 g). Kawali had the average per panicle weight and grain weight per panicle of 77.67 and 58.02 g, respectively. All of the sorghum lines had panicle weights and grain weights per panicle that were not significantly different from Numbu (Table 4).

1000-grain weight is a character that shows the size of sorghum seeds. High 1000-grain weight indicates a large grain size. The mean 1000-grain weight of sorghum lines ranged from 26.54 g to 35.54 g. Numbu had the largest 1000-grain weight of 35.54 g, whereas the smallest 1000-grain weight belonged to line N/UP-118-7 with a weight of 26.54 g. Lines N/UP-4-3, N/UP-124-7, N/UP-139-1, N/UP-156-8, and N/UP-48-2 had 1000-grain weights that were significantly greater than Kawali (Table 4).

Days to flowering were determined when 80% of the plants have flowered. The days to flowering and days to maturity of the sorghum lines were 65.00–74.33 and 95.00–103.67 DAS, respectively. The days to flowering and days to maturity of the sorghum lines were significantly longer than those of Kawali. The grain-filling period starts from the first day of flowering until the grains are physiologically mature, which is marked by the black layer at the base of the embryo. The sorghum lines N/UP-4-3, N/UP-124-7, N/UP-166-6, and N/UP-89-3 have significantly longer grain-filling periods than Numbu and Kawali (Table 4).

The productivity of sorghum lines was between 2.22–4.08 tons ha^{-1}. Numbu had the highest average productivity of 4.08 tons ha^{-1}. The productivity of lines N/UP-4-3, N/UP-118-3, N/UP-118-7, N/UP-159-9, N/UP-17-10, and N/UP-89-3 was significantly higher than that of the national variety Kawali (2.71 tons ha^{-1}).

Table 5. Combined analysis of variance for the disease severity of sorghum leaf rust at different times of assessment.

Time of assessment	Mean squares		
	Environment (E)	Genotype (G)	G × E
60 days after sowing	1936.88**	229.13*	62.33**
70 days after sowing	509.67	803.44**	125.44*
80 days after sowing	10482.31**	1682.71**	340.29**

**, * = Significant at 1% and 5% of probability, respectively.

Table 6. Estimation of variance components and broad sense heritability for disease severity of sorghum leaf rust at different times of assessment.

Time of assessment	σ^2_g	σ^2_e	σ^2_{ge}	σ^2_p	h^2_{bs} (%)
60 days after sowing	27.80	9.26	17.69	38.19	73
70 days after sowing	113.00	57.03	22.80	133.91	84
80 days after sowing	223.70	39.75	100.31	280.41	80

σ^2g = genotypic variance; σ^2e = environmental variance; σ^2ge = G × E variance; σ^2p = phenotypic variance, h^2(bs) = broad sense heritability.

Resistance to leaf rust in sorghum lines

The combined analysis of variance was significant for environment, genotypes, and their interactions on the disease severity of leaf rust (Table 5). The estimated value of broad-sense heritability (h^2_{bs}) of leaf rust disease severity in sorghum at 60, 70, and 80 DAS were high and were 73%, 84%, 80%, respectively, which were classified as high heritability values (Table 6). If the heritability value was high, the level of the resistance of the lines between locations was not expected to change considerably.

The significant G × E effect resulted in the differential leaf rust disease severity of sorghum lines (Table 7). The line with the lowest disease severity in Maros at 60 DAS was N/UP-48-2, whereas the line with the lowest disease severity in Bogor was N/UP-159-9. In Maros, all lines had higher disease severity than the check varieties, Numbu, and Kawali. However, in Bogor, one line, N/UP-159-9, was not significantly different from the check varieties. The assessment in Maros at 70 DAS showed that the severity of leaf rust disease did not significantly differ among sorghum lines. However, line N/UP-48-2 showed the lowest rust disease severity value in Bogor. Line N/UP-48-2 also showed the lowest rust disease severity value at 80 DAS and was not significantly different from Numbu and Kawali.

The assessment at 80 DAS was determined as a reference in determining the criteria for resistance because the percentage of leaf rust disease severity in susceptible varieties was greater than 60%. This result indicated that the inoculum was spread evenly and was available in sufficient quantities to infect sorghum plants in the test environment.

The determination of the resistance level of the tested lines was conducted based on the percentage of disease severity. Numbu was considered as resistant in Maros and Bogor, whereas Kawali was classified as moderately resistant in Maros (Table 8). The susceptible varieties used as SR plants in the Maros test environment showed high disease severity such that they could function as a source of inoculum. Based on the results of leaf rust scoring at the planting location in Maros, no lines were identified as resistant. The test environment in Bogor revealed that the availability of natural inocula was sufficient for testing resistance to leaf rust disease. Among the sorghum lines tested in Bogor, only line N/UP-166-6 was resistant to leaf rust disease.

Table 7. Variation in the disease severity of sorghum leaf rust in Maros and Bogor.

Genotype	60 DAS			70 DAS			80 DAS		
	Maros	Bogor	Means	Maros	Bogor	Means	Maros	Bogor	Means
N/UP-4-3	23.44^{ab}	6.25^{cd}	14.84^{bc}	36.15^{a}	31.77^{bc}	33.96^{abcd}	70.73^{bc}	45.83^{ab}	58.28^{ab}
N/UP-118-3	18.75^{bc}	15.63^{ab}	17.19^{ab}	45.00^{a}	35.94^{ab}	40.47^{ab}	69.69^{bc}	43.23^{ab}	56.46^{b}
N/UP-118-7	23.96^{ab}	7.81^{cd}	15.89^{abc}	35.52^{a}	35.94^{ab}	35.73^{abcd}	64.90^{cd}	50.00^{ab}	57.45^{ab}
N/UP-139-5	29.17^{a}	8.33^{cd}	18.75^{ab}	40.00^{a}	34.90^{b}	37.45^{abcd}	75.83^{ab}	38.02^{b}	56.93^{ab}
N/UP-156-8	20.31^{b}	5.73^{d}	13.02^{cd}	32.40^{a}	31.25^{bc}	31.82^{bcd}	61.25^{d}	40.10^{ab}	50.68^{bc}
N/UP-159-9	14.06^{cd}	4.69^{de}	9.38^{d}	26.67^{a}	29.69^{c}	28.18^{d}	52.92^{e}	41.67^{ab}	47.29^{c}
N/UP-17-10	20.83^{b}	17.19^{a}	19.01^{a}	45.52^{a}	40.10^{a}	42.81^{a}	77.19^{ab}	53.13^{a}	65.16^{a}
N/UP-48-2	13.02^{d}	6.25^{cd}	9.64^{d}	45.10^{a}	15.10^{d}	30.10^{cd}	71.67^{abc}	20.83^{c}	46.25^{c}
N/UP-82-3	23.96^{ab}	11.46^{bc}	17.71^{ab}	39.58^{a}	34.38^{bc}	36.98^{abcd}	71.56^{abc}	44.79^{ab}	58.18^{ab}
N/UP-89-3	22.92^{b}	8.33^{cd}	15.63^{abc}	45.63^{a}	33.33^{bc}	39.48^{abc}	80.10^{a}	36.98^{b}	58.54^{ab}
Numbu	1.56^{e}	0.00^{e}	0.78^{e}	6.35^{b}	8.85^{e}	8.49^{e}	7.08^{g}	11.98^{c}	9.53^{e}
Kawali	4.17^{e}	0.00^{e}	2.08^{e}	7.08^{b}	9.90^{e}	7.60^{e}	27.29^{f}	14.06^{c}	20.68^{d}
Means	18.01	7.64	12.83	33.75	28.43	31.09	60.85	36.72	48.78

Number in the same column followed by the same letter are not significant at DMRT 5%.

Table 8. Criteria for resistance to leaf rust in sorghum lines at 80 DAS in Maros and Bogor, Indonesia.

Genotype	Maros	Bogor
N/UP-4-3	Highly susceptible	Susceptible
N/UP-118-3	Highly susceptible	Susceptible
N/UP-118-7	Highly susceptible	Susceptible
N/UP-139-5	Highly susceptible	Moderately resistant
N/UP-156-8	Highly susceptible	Susceptible
N/UP-159-9	Susceptible	Susceptible
N/UP-17-10	Highly susceptible	Susceptible
N/UP-48-2	Highly susceptible	Moderately resistant
N/UP-82-3	Highly susceptible	Susceptible
N/UP-89-3	Highly susceptible	Moderately resistant
N/UP-124-7	-	Susceptible
N/UP-139-1	-	Susceptible
N/UP-166-6	-	Resistant
Kawali	Resistant	Resistant
Numbu	Moderately resistant	Resistant
Super 1	Susceptible	-
Super 2	Highly susceptible	-

- = Not planted.

Table 9. Changes in the resistance ranking of leaf rust disease in Maros and Bogor.

Rangking	Maros	Bogor
1	Kawali	Kawali
2	Numbu	Numbu
3	N/UP-159-9	N/UP-48-2
4	N/UP-156-8	N/UP-89-3
5	N/UP-118-7	N/UP-139-5
6	N/UP-118-3	N/UP-156-8
7	N/UP-004-3	N/UP-159-9
8	N/UP-82-3	N/UP-118-3
9	N/UP-48-2	N/UP-82-3
10	N/UP-139-5	N/UP-004-3
11	N/UP-17-10	N/UP-118-7
12	N/UP-89-3	N/UP-17-10

Based on the results of this study, it was also known that the level of resistance of sorghum lines to leaf rust disease was influenced by G × E interactions. This caused a change in the resistance ranking of the leaf rust disease in two different locations (Table 9). Only resistant varieties had consistent responses in both environments.

Correlation between leaf rust severity and agronomic traits

The results of the correlation analysis of several agronomic traits with leaf rust disease severity in Bogor showed that disease severity at 60, 70, and 80 DAS did not have a significant correlation with productivity (Table 10). The disease severity of leaf rust had a significant negative correlation only with panicle length. This indicated that high disease severity would be followed by a decrease in panicle length.

The disease severity of leaf rust at 60 DAS was positively correlated with disease severity at 70 and 80 DAS (Table 10). The sorghum lines lacked a recovery mechanism to inhibit or reduce the growth of pathogens as the plant matures. This was thought to be the cause for the response of sorghum lines ranging from susceptible to very susceptible.

DISCUSSION

The improvement in yield potential is the main goal of sorghum plant breeding followed by the improvement in plant stature and seed color. Genotypes with shorter stature and white seeds are preferred as a food source. The sorghum lines tested in this study are advanced breeding lines selected from a segregating population following a cross between Numbu and UPCA S1. Numbu has a high plant height and good general combining ability for plant height (Rini *et al.,* 2017), and it will have tall progenies. However, Numbu has white grains and low tannin content (Trikoesoemaningtyas *et al.,* 2015) making it very suitable as a food source.

Plant height is associated with resistance to lodging. Short plants tend to be more resistant to lodging. However, plants with short stature also tend to have low yields. The results of this study indicated that a decrease in plant height caused a

Table 10. Pearson's correlation coefficients between leaf rust severity and agronomic traits in Bogor.

Traits	PH	SW	PL	PW	GW	TGW	DF	DM	GF	DS1	DS2	DS3	Y
PH	1.00												
SW	0.39	1.00											
PL	-0.27	-0.08	1.00										
PW	0.76*	0.50	0.20	1.00									
GW	0.71*	0.28	0.28	0.96*	1.00								
TGW	0.38	-0.44	-0.14	0.21	0.29	1.00							
DF	0.59*	0.31	-0.60*	0.17	0.06	0.08	1.00						
DM	0.30	0.07	-0.86*	-0.18	-0.22	-0.02	0.75*	1.00					
GF	-0.48	-0.37	-0.22	-0.48	-0.36	-0.14	-0.51	0.19	1.00				
DS1	0.40	0.34	-0.57*	0.04	-0.10	-0.04	0.31	0.34	-0.03	1.00			
DS2	0.32	0.31	-0.70*	0.05	-0.06	-0.20	0.40	0.52*	0.09	0.80*	1.00		
DS3	0.41	0.35	-0.61*	0.22	0.11	-0.19	0.36	0.46	0.06	0.77*	0.97*	1.00	
Y	0.81*	0.50	0.07	0.94*	0.92*	0.22	0.25	0.01	-0.35	0.06	0.02	0.18	1.00

* = significant at 5%; PH = plant height (cm); SW = stem width (mm); PL =panicle length (cm); PW = panicle weight (g); GW = grain weight per panicle (g);TGW = 1000-grain weigth (g); DF = days to flowering (DAS); DM = days to maturity (DAS); GF = grain-filling period (days); DS1 = disease severity at 60 DAS (%); DS2 = disease severity at 70 DAS (%); DS3 = disease severity at 80 DAS (%); Y = productivity (tons ha^{-1}).

decrease in yield marked by a positive correlation coefficient. Some studies also reveal similar results, wherein a decrease in plant height has consequences in decreasing yield (Reddy and Jabeen *et al.,* 2016; Hundekar *et al.,* 2016; Mofokeng *et al.,* 2019). In this study, two lines, N/UP-4-3 and N/UP-118-3, were selected because their yields were not significantly different from that of Numbu with white grains, and both lines have shorter stature than Numbu.

The development of resistant varieties is aimed to prevent a decrease in yield due to disease infection. Yield losses in sorghum due to leaf rust disease in Indonesia have never been reported. However, other studies reported that yield loss in sorghum due to leaf rust infection could reach 65% in susceptible plants (Bandyopadhyay, 2000). Plants susceptible to leaf rust tend to be susceptible to other sorghum diseases, especially those that are also caused by fungi (Das and Rajendrakumar, 2016), such as stem rot and anthracnose (Frederiksen, 1980; Mohan *et al.,* 2010). Based on this information, sorghum resistance to leaf rust has become a requirement for the release of sorghum varieties in Indonesia.

This study succeeded in identifying the disease severity of leaf rust in sorghum lines in two different locations in Indonesia. The results showed that leaf rust infections were not correlated with yield in sorghum lines tested. This is indicated by some very susceptible lines that have productivity that was not significantly different from Numbu, which is the resistant check variety (Table 4). Up till now, leaf rust in sorghum has not caused significant yield loss in Indonesia.

Almost all lines tested showed a high disease severity of up to >40% at 80 DAS. Two national varieties

(Numbu and Kawali) showed a low percentage of disease severity in both locations, and line N/UP-166-6 showed a low percentage of leaf rust disease severity in Bogor. The sorghum lines were moderately resistant to very susceptible in the two environments, except for line N/UP-166-6, which was classified as resistant in Bogor and was not observed in Maros.

Leaf rust disease resistance is polygenic and is thus strongly influenced by the environment (Mohan *et al*. 2010). In this study, we found that disease severity in sorghum lines was influenced by genotype, environment, and G × E interactions. The effect of G × E interactions on the disease severity of leaf rust was also reported in several other plants (Rosewarne *et al*. 2008; Mohan *et al*. 2010; Mboup *et al*. 2012; Sillero *et al*. 2017). However, disease severity is highly influenced by genetic factors as shown by high heritability values in all observation periods, so that it is very likely to select resistant genotypes in early and advanced generations. The same result was reported by Cheng *et al.* (2019) and Zeng *et al.* (2019) for stripe rust in wheat with heritability values ranging from 85% to 91%. The presence of genetic variance in the disease severity of leaf rust in sorghum was also reported by White *et al.* (2015) in 10 sorghum genotypes and by Mu *et al.* (2019) in 60 sorghum genotypes.

This study found that the severity of leaf rust was influenced by G × E that changed the value of leaf rust disease severity between test sites. Numbu and Kawali, which were used as check varieties, were ranked the same in both environments. On the other hand, there was a change in the resistance ranking for other sorghum lines. Lines N-UP-139-5, N/UP-48-2, and N/UP-89-3 were very susceptible in Maros but were moderately resistant in Bogor.

The sorghum lines tested in Bogor were more resistant than those in Maros, whereas the responses of Kawali and line N/UP-159-9 did not change in the two environments (Table 8). This shows significant G × E interactions caused by Kawali and line N/UP-159-9. Other sorghum lines in Bogor had the same pattern, which shows a better resistance response because the resistance response is more influenced by the environment than the G × E interaction. The observed resistance was horizontal resistance as indicated by the response of sorghum lines that were more resistant or improved when tested in Bogor. Horizontal resistance or field resistance is characterized by greater environmental influences; thus, changes in host plant response follow changes in pathogen virulence in the test environment (Vanderplank 1984). It is thought that the environmental factors and methods of infection caused the sorghum lines tested in Bogor to become more resistant. The smaller number of pathogens due to host plant infections occurred naturally, and the aggressiveness of pathogens was weakened due to less optimal environmental conditions for the growth of pathogens in Bogor.

Rainfall and the number of rainy days in Bogor were higher, but the percentage of leaf rust disease severity was higher at Maros (Tabel 7), which had an average temperature of 28 °C, an average humidity of 76%, an average rainfall of 220 mm (medium), and an average of 13 rainy days. This is presumably due to the availability of more pathogenic inocula in Maros because of the inoculation of pathogens through SR plants. The SR plants were inoculated withurediniospore suspension and acted as the source of donor inoculum for the tested sorghum plants. The multiple inocula in SR plants spread to sorghum plants in the screening plot. The availability of excessive or abundant inocula in screening plots is a prerequisite to ensure that the plant is resistant and not because it escapes from disease (Sharma, 1978). In contrast to the results reported by Mohan *et al.* (2010), leaf rust infection was found only in environments where pathogen infections occur naturally (without SR plants), whereas anthracnose infection is found in environments with inoculation and without inoculation of inocula.

The different endurance responses are thought to be due to differences in the amount of inocula in the field and leaf rust pathotype. This study was conducted in two environments when conditions supported leaf rust infection. As a result, the possibility of plant infection with more than one pathotype can be a cause of the difference in disease severity (White *et al.,* 2015).

The results of the correlation analysis also showed that disease severity at 60, 70, and 80 DAS lacked a significant correlation with productivity (Table 10). Damage to leaves by infection with the leaf rust pathogen in the generative period did not affect the filling of sorghum seeds. Sorghum plants, in addition to storing photosynthate in panicles, also have the ability to stored photosynthate in their stems (similar to sugar cane) (Qazi *et al*. 2012). Stems act as a site of photosynthate accumulation during the vegetative period. Thus, sorghum seed filling does not always rely on photosynthate from flag leaves during the generative period. Given that photosynthate has accumulated in plant stems since the vegetative phase, the decrease in the amount of photosynthate during generative due to pathogenic infections in leaf tissue has no effect on sorghum seed filling.

The lines used in this study have not yet been tested for their resistance to rust. In addition, rust disease has never been found in the growing season before because the purpose of the sorghum breeding effort that has been done is to improve productivity, and these lines were planted in an optimal environment. Therefore, the resistance gene to rust disease was not fixed in this generation. Selection for resistance conducted in later generations has little chance of obtaining resistant lines. The characteristic of leaf rust resistance in sorghum is polygenic. Thus, it is recommended that selection to obtain resistant lines should be carried out since the F4 generation.

Numerous studies have confirmed the polygenic nature of rust resistance in sorghum (Tao *et al.,* 1998; McIntyre *et al.,* 2004; McIntyre *et al.,* 2005; Mohan *et al.,* 2010). McIntyre *et al.* (2004) reported that the Rp1-D gene that controls rust resistance in corn has been found in sorghum and is present in the QTL that was mapped previously by Tao *et al.* (1998). McIntyre *et al.* (2005) successfully mapped 17 out of 31 sugarcane resistance gene analogues in the sorghum genome. Mohan *et al.* (2010) identified two QTL related to sorghum rust resistance at the SBI-06 locus. One of these QTL shares a similarity with the Rp1-D gene in corn.

CONCLUSION

The leaf rust disease of sorghum lines was influenced by G × E interactions. The sorghum lines in Maros were susceptible to very susceptible. Three lines, namely, N/UP-139-5, N/UP-48-2, and N/UP-89-3, were classified as moderately resistant in Bogor, whereas line N/UP-166-6 was resistant. The results of this study can help breeders determine the plant age that is the most beneficial for the selection of leaf rust resistance. The observation of disease severity for the selection of resistant lines can be carried out at the age of 70 DAS because of the small effect of environment and G × E interaction. The infection of leaf rust in sorghum was shown to have no correlation with yield in sorghum lines.

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