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Research Article
Field Evaluation of Late Leaf Spot and Leaf Rust Resistance and the Associated Yield Losses in Indonesian Groundnut Genotypes

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Abstract

Background and Objective: Groundnut is the second most important pulse crop in Indonesia and the most important one in the semi-arid region of East Nusa Tenggara province. Productivity of this crop in the farmer level is low due to many factors. Late leaf spot and leaf rust are the most significant diseases of groundnut that can cause considerable yield losses. Information on disease resistance and associated yield losses in Indonesian groundnut genotypes is lacking. The present study aimed at elucidating late leaf spot and leaf rust resistance levels of Indonesian groundnut genotypes and assessing yield losses caused by the diseases. **Materials and Methods:** Five groundnut genotypes were evaluated in three environmental sets, i.e., protected, late leaf spot and leaf rust plots. Observed variables included incubation period, disease severity, AUDPC, defoliation (%), number of pustules and pod yields. All data, except for disease severity score were subjected to variance analysis, followed by DMRT *post hoc* test and correlation analysis. The disease severity score was analyzed using Kruskal Wallis procedure followed by Mann Whitney U-test. **Results:** Research results showed significant variations in observed variables of leaf spot and leaf rust diseases and the associated yield losses in the tested groundnut genotypes. Local Rote was 'moderately resistant' while the Indonesian released varieties were 'susceptible' and 'highly susceptible' to late leaf spot. The tested genotypes showed 'susceptible' and 'highly susceptible' reactions to leaf rust. Pod yield loss caused by late leaf spot and leaf rust diseases ranged from, respectively, 15-57 and 23-57%. **Conclusion:** Only Local Rote was moderately resistant to late leaf spot and the others were susceptible and none of the genotypes was resistant/moderately resistant to leaf rust. Yield lost caused by late leaf spot was low to high levels while that of leaf rust was moderate to high levels.

Key words: Resistance, late leaf spot, leaf rust, yield loss, groundnut

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is the second most important pulse crop in Indonesia and the first most important in the Indonesian semi-arid region of East Nusa Tenggara (ENT) Province. Groundnut is used by the farmers in ENT province as a source of food and cash income. The average productivity of the crop at the ENT province level was low ranging from 0.87-1.02 t ha^{-1} dry pods while that at the national level was 1.28-1.33 t ha^{-1} dry pods[1]. This low productivity is caused by various factors such as poor cultivation practices, drought stress, foliar diseases and cultivation of low yielding varieties.

Late leaf spot (*Cercosporidium personatum* Berk and Curtis Deighton) and leaf rust (*Puccinia arachidis* Speg.) are the most destructive foliar diseases of groundnut in Indonesia[2]. Growing groundnut varieties that are resistant to both diseases is crucial since yield losses caused by the diseases are high, ranging from 10-60%[3-7]. A yield loss of up to 70% was observed when the two diseases occurred together[8]. In the greenhouse condition, yield losses caused by late leaf spot and leaf rust have recently been reported to reach 61-85%[9]. More recently, Mohammed *et al.*[10] observed a highly significant and negative correlation between late leaf spot disease severity and pod yield but the percentage of the yield loss was not recorded. Many studies have been dealing with late leaf spot and leaf rust resistance but their associated percentage yield losses are limitedly reported.

A number of superior groundnut varieties have been released by the Indonesian Legumes and Tuber Crops Institute but those are limitedly available to the farmers, especially in the ENT province. Meanwhile, most of the local cultivars are low yielding. Kacang Rote (Local Rote) is a local cultivar of Rote Ndao district of ENT province that has been well known for its large seed size, high yield and tolerance to drought[11]. This local cultivar has the potential to be released as a superior variety with a specific adaptation to the semi-arid region of ENT province. However, information on this cultivars' resistance to biotic stresses such as late leaf spot and leaf rust diseases and the associated yield losses are presently unavailable. Disease resistance levels of several Indonesian released varieties have been provided in their varietal descriptions[12] but information on their yield losses caused by the diseases in the field is lacking. The present study aimed at elucidating the resistance levels of Indonesian groundnut genotypes to leaf spot and leaf rust diseases and assessing yield losses caused by the diseases.

MATERIAL AND METHODS

Experimental site: This study was conducted in the farmer's field in Kaniti Village, Kupang district, East Nusa Tenggara province (10°09'38.92"S latitude and 123°41'13.56"E longitude, 47 m above sea level) during the rainy season (February-May) 2014. Soil type of the experimental field was Vertisol (Grumosol: USDA). The previous crop was a fallow of maize (*Zea mays* L.) plant. Monthly rain fall intensity during the experiment averaged about 34-420 mm while the daily mean temperature was 26.8-27.8°C and relative humidity was 77-84%.

Experimental design, plant materials and cultivation: The current experiment was laid out in a randomized complete block design with groundnut genotype as treatment, i.e., Local Rote, Gajah, Jerapah, Bison and Kancil. The four Indonesian released varieties were kindly provided by Indonesian Legumes and Tuber Crops Research Institute while the Local Rote Cultivar was provided by the Agriculture and Estate Department of ENT province. Three experimental sets were laid out in the field, one for un-inoculated/protected plots, one for late leaf spot inoculation plots and the other for leaf rust inoculation plots. The three experimental sets were separated each other 10 m away and bordered with five rows (70 cm$\times$20 cm planting space) of maize plants. Each experimental set consisted of five groundnut genotypes as treatments; each was three replicates. Each genotype was sown in plots of 3 m$\times$2 m size with 40 cm$\times$40 cm plant spacing. Two seeds were grown in each planting hole, but only one plant was retained until harvest. The plants were maintained under the standard groundnut cultivation technique.

Plant inoculation: Late leaf spot (*C. personatum*) and leaf rust (*P. arachidis*) inoculations were carried out at 21 days after planting (DAP). The *C. personatum* was obtained from infected leaf tissue in the field. Pieces of infected leaf tissue were surface sterilized and then planted on a Potato Dextrose Agar (PDA) medium for six days, followed by isolation and identification of the pathogen. Two week old isolated *C. personatum* was then used to prepare the inoculum solution. *P. arachidis* inoculation was carried out using spores obtained from freshly infected leaf tissues. The infected leaf tissues were crushed with a mortar and the resulting filtrate was filtered and added with distilled water for preparation of inoculum solution. A conidial concentration of 1.0×10^5 conidia mL^{-1} was used for plant inoculations by using an aerosol spray bottle. The protected plots were not inoculated with either *C. personatum* or *P. arachidis* and were sprayed in a two weekly interval from 14 days after planting (DAP) using fungicide Benlate 50 WP (Benomyl) at a rate of 1.0 kg ha^{-1} to prevent the onset of both leaf spots and leaf rust diseases. Benlate 50 WP was also used once to spray the plants in the inoculated plots at two weeks after planting or one week before inculcation with inoculum of the two pathogens at 21 DAP.

Observation and data analysis: Observed variables included disease severity, leaf defoliation (%) (for leaf spot only), number of pustules cm^{-2} leaf area (for leaf rust only) and dry pod yield. The incubation period was observed daily from day one after inoculation while disease severity was observed weekly from 35-77 DAP. Defoliation (%) and number of pustules cm^{-2} leaf area were recorded one week after the last disease severity assessment (84 DAP). Dry pod yield was observed after harvest.

The incubation period was measured as number of days from inoculation to the appearance of the first symptom (lesion)[13]. Disease severities (disease scores) of both late leaf spot and leaf rust and resistance levels of the evaluated genotypes were assessed using the modified ICRISAT 9 point scale and pictorial key[14], where infected leaf area (%) was: 1: No disease (0%), 2: 1-5%, 3: 6-10%, 4: 11-20%, 5: 21-30%, 6: 31-40%, 7: 41-60%, 8: 61-80% and 9: 81-100%. The groundnut genotype's resistance levels to both diseases were classified as 'Resistant' (disease score 1), 'Moderately Resistant' (disease scores 2-3), 'Moderately Susceptible' (diseases score 4-5), 'Susceptible' (disease scores 6-7) and "Highly Susceptible' (disease scores 8-9). The resistance levels were classified based on the disease severity recorded at the last assessment. Assessments of disease severity, percent defoliation and number of pustules cm^{-2} leaf area were done on 10 plants in the center two rows of each plot while pod yield was observed by weighing dry pods from of all plants in each plot and expressed in kg ha^{-1}. The disease severity scores were converted to Disease Severity Index (DSI)[15], which then used to calculate the area under the disease progress curve (AUDPC)[16,17]. Defoliation (%) was calculated as the ratio of leaflets lost to the total leaflets on the main stem of the ten previous plants. It was computed as follows[18]:

$$\text{Defoliation (\%)} = \frac{\text{Number of 'leaflets' lost}}{\text{Total 'leaflets' lost}} \times 100$$

Number of pustules cm^{-2} leaf area was determined by counting the number of pustules in each leaf of the main stem, which then converted into number of pustules cm^{-2} leaf area. Yield loss caused by either late leaf spot or leaf rust was calculated with the following equation:

$$L = \frac{T\text{-}U}{T} \times 100$$

Where:
L = Loss (%)
T = Yield in kg ha^{-1} of the protected plot
U = Yield in kg ha^{-1} of the diseased plot[19]

In each experimental set, the groundnut genotype effects on disease severity score (ordinal data) was determined using Kruskal-Wallis procedure and the means were separated using Mann Whitney U-test. Meanwhile the treatment effects on AUDPC, incubation period, defoliation (%), number of pustules cm^{-2}, pod yield and pod yield loss were assessed using one-way analysis of variance and the Duncan's Multiple Range Test (DMRT) at 5% level of significance was used to detect the difference between the treatment means. It also carried out correlation analysis to assess the relationships among the observed variables. All data analysis was performed using Genstat version[20] 12.1.

RESULTS

Incubation period: Symptoms of leaf spot and leaf rust in the present study started to appear over one week after inoculation. There was no significant effect ($p>0.05$) of groundnut genotype on incubation period of both late leaf spot and leaf rust. Incubation period of late leaf spot ranged from 9.3-10.3 days after inoculation (DAI) while that of leaf rust ranged from 11.3-12.7 DAI.

Disease severity and resistance level: Although the control (protected) plots were sprayed with fungicide, there were still a low level of both leaf spot and leaf rust disease infections (1-5% severity), depending on the groundnut genotypes. Weekly disease severity index (%) was used to construct the disease progress curves as presented in Fig. 1. At the first assessments (first and second weeks), late leaf spot severities were almost similar among genotypes but it started to separate at the 3rd week and became more evident in the following weeks, where the genotypes deviated clearly into two groups, i.e., Local Rote alone with a lower disease severity index in one group and the other four Indonesian released varieties with higher disease severity index at another group (Fig. 1a).

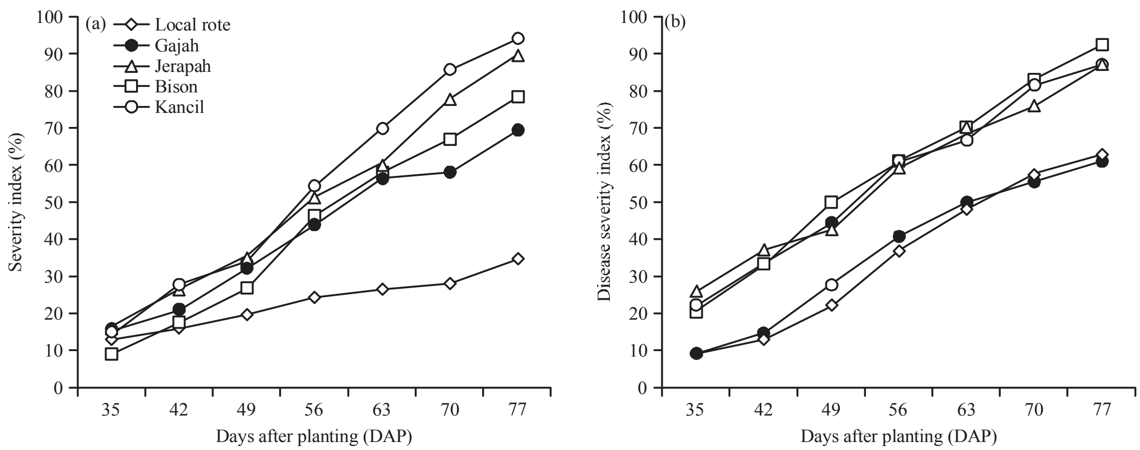

Fig. 1(a-b): Disease progress curves of (a) Late leaf spot and (b) Leaf rust in five Indonesian groundnut genotypes. Disease severity index was converted from the disease score of the modified ICRISAT 9 rating scale for both late leaf spot and leaf rust

Table 1: Late leaf spot and leaf rust disease severity, AUDPC, defoliation (%) and number of pustules cm^{-2} leaf area of Indonesian groundnut genotypes

	Late leaf spot				Leaf rust			
Groundnut genotype	Severity	AUDPC (days %)	Defoliation (%)*	Resistance level	Severity	AUDPC (days %)	Number of pustules cm^{-2}	Resistance level
Local rote	3.1^{a}	750.6^{a}	76.2^{a}	MR	5.8^{a}	1545.8^{a}	4.9^{b}	S
Gajah	6.3^{b}	1774.4^{b}	92.6^{b}	S	7.8^{b}	2391.7^{b}	5.4^{c}	HS
Jerapah	8.1^{bc}	2126.7^{c}	95.2^{c}	HS	7.8^{b}	2378.7^{b}	5.5^{c}	HS
Bison	7.1^{b}	1813.1^{b}	93.7^{b}	S	8.3^{b}	2482.4^{b}	6.5^{d}	HS
Kancil	8.8^{c}	2280.2^{c}	97.4^{c}	HS	5.5^{a}	1518.5^{a}	4.3^{a}	S

Disease severity score was subjected to Kruskal-wallis analysis while AUDPC of disease severity index, defoliation (%) and number of pustules cm^{-2} were analyzed using one-way ANOVA. *Defoliation (%) data were subjected to square root transformation prior to analysis of variance. MS: Moderately resistant, S: Susceptible, HS: Highly susceptible. Means under the same column followed by the same letter(s) are not significantly different by Mann Whitney U-test and DMRT (5%)

The data in Fig. 1b showed that leaf rust disease severities were divided into two groups with Local Rote and Kancil in the first group (lower disease scores) while Gajah, Jerapah and Bison were in the second group (higher disease scores). The same situation applied throughout the observation period.

Kruskal Wallis analysis revealed that mean disease score of late leaf spot at the last assessment differed significantly ($p<0.05$) among the genotypes (Table 1). Local Rote exhibited the lowest late leaf spot disease score with an average score of 3.1. Kancil, on the other hand, exhibited the highest late leaf spot disease severity score (8.8) but was not statistically different from Jerapah.

Like disease severity score, the groundnut genotypes also showed significant differences ($p<0.05$) in both area under the disease progress curve (AUDPC) of disease severity index and defoliation (%) of late leaf spot. Local Rote showed the lowest AUDPC (750.6% days) while Kancil showed the highest AUDPC (2280.2% days) but did not differ from that of Jerapah (2126.7% days), indicating differential late leaf spot disease development over time among the genotypes. Similar with disease severity and AUDPC of disease severity index, defoliation (%) caused by late leaf spot also differed significantly ($p<0.05$) among the genotypes. Local Rote suffered about 76% defoliation while other genotypes suffered significantly higher defoliation (>90%).

Resistance level data presented in Table 1 showed that only Local Rote exhibited a 'moderately resistant' reaction while others showed lower resistance levels (susceptible and highly susceptible) reactions. The higher resistance level of Local Rote was in accordance with its lower disease severity score, lower AUDPC of disease severity index and lower percent defoliation as compared to other genotypes. On the other hand, the lower resistance level of Kancil was in line with its higher records in disease score, AUDPC of disease severity index and percent defoliation.

The study results also revealed that leaf rust disease severity and AUDPC were also varied significantly (Table 1). Kancil (5.5) and Local Rote showed the lowest leaf rust score. AUDPC of rust disease severity index also differed significantly ($p<0.05$) among the genotypes with Kancil and Local Rote consistently showed the lowest AUDPC (1518.5% days and 1545.8% days, respectively) as with its lowest leaf rust severity.

Table 2: Pod yield and pod yield loss of Indonesian groundnut genotypes under protected, leaf spot and leaf rust plots

Groundnut genotype	Pod yield (kg ha^{-1})			Pod yield loss (%)*	
	Protected plots	LLS plots	LR plots	LLS plots	LR plots
Local rote	2733^{d}	2318^{c}	1950^{d}	15.0^{a}	28.6^{b}
Gajah	2324^{c}	1234^{b}	1429^{c}	46.9^{c}	38.5^{c}
Jerapah	2245^{c}	975^{a}	1350^{c}	56.5^{c}	39.7^{c}
Bison	1718^{b}	1230^{b}	748^{a}	28.4^{b}	56.6^{d}
Kancil	1596^{a}	867^{a}	1228^{b}	45.7^{c}	23.1^{a}

LLS: Late leaf spot, LR: Leaf rust. *Pod yield loss (%) data were subjected to archin transformation prior to analysis of variance. Means under the same column followed by the same letter(s) are not significantly different at 5% DMRT

Table 3: Results of correlation analysis involving disease and yield variables of Indonesian groundnut genotypes

	Leaf spot		
	Defoliation	Pod yield	Pod yield loss
Disease severity score	0.98*	-0.98*	0.50
AUDPC	0.98*	-0.99*	0.50
Defoliation		-0.98*	0.50
	Leaf rust		
	Number of pustules	Pod yield	Pod yield loss
Disease severity score	0.97*	-0.34	0.97*
AUDPC	0.90*	-0.56	0.90*
Number of pustules		-0.40	0.99*

*Significant at t-test 0.05 level of significance

In leaf rust, number of pustules cm^{-2} leaf area varied substantially ($p<0.05$) among the genotypes, ranged from 4.3-6.5 pustules. Kancil (4.3) showed the lowest pustules cm^{-2}. The lowest number of pustules cm^{-2} of Kancil was well confirmed in its significantly lower disease score and AUDPC.

Despite their considerable variation in leaf rust disease variables, the five genotypes were classified only into two resistance categories, i.e., 'susceptible' (Kancil and Local Rote) and 'highly susceptible' (Gajah, Jerapah, Bison). No resistant reaction was observed in the present study (Table 1).

Yield performance and yield loss: Pod yield reported in this paper was converted from dry pod yield/6 m^{2} plot into dry pod yield ha^{-1} before analysis of variance. Mean pod yield of tested groundnut genotypes were significantly different (Table 2). Of the five genotypes evaluated, Local Rote performed the best in all the three experimental sets, i.e., 2733 kg ha^{-1} in protected plots, 2318 kg ha^{-1} in late leaf spot plots and 1950 kg ha^{-1} in leaf rust plots. Pod yields of Local Rote in leaf rust plots (1950 kg ha^{-1}) was also significantly higher than other genotypes.

Percentages of pod yield losses caused by both diseases were presented in Table 2. Like pod yield, percentage of pod yield loss caused by either late leaf spot or leaf rust also significantly differed ($p<0.05$) among genotypes. In late leaf spot plots, pod yield loss of Local Rote was the lowest (15.0%), which was significantly different from other genotypes, i.e., Bison (28%), Kancil (45.7%), Gajah (46.9%) and Jerapah (56.6%).

Kancil suffered the lowest yield loss (23.1%) in leaf rust plots, followed by Local Rote with a moderate yield loss (28.6%). Gajah, Jerapah and Bison suffered, respectively, 38.5, 39.7 and 56.6% pod yield losses.

Correlation among observed variables: Observed variables included in the correlation analysis were disease severity score, AUDPC of disease severity index, defoliation (%), number of pustules, pod yield and pod yield loss (Table 3). Late leaf spot disease variables such as disease severity score, AUDPC and defoliation (%) were significantly and negatively correlated with pod yield but their correlation with pod yield loss were not significant. In contrast to late leaf spot, leaf rust disease variables such as disease severity score, AUDPC and number of pustules cm^{-2}were not significantly correlated with pod yield but were significantly and positively correlated with pod yield loss. This may indicated differential resistance or tolerance mechanism of the genotypes against both diseases.

DISCUSSION

The study results provide invaluable information on resistance levels, yield performances and yield losses caused by late leaf spot and leaf rust diseases of the tested groundnut genotypes. The genotypes exhibited significant different disease variables, except incubation period, which may indicate differential resistance response to late leaf spot and leaf rust diseases. Significant variation among genotypes in late leaf spot disease variables had been previously reported[10,21] while that in leaf rust was lacking. Meanwhile, the observed insignificant incubation period of either late leaf spot or leaf rust agrees with the previous findings[9,13,22].

Only Local Rote was 'moderately resistant' to late leaf spot, characterized by its low disease severity, while other genotypes were 'susceptible' and 'highly susceptible'. Lower late leaf spot severity of Local Rote was in line with its lower records in AUDPC and defoliation (%). Lower AUDPC indicates lower disease development rate while lower defoliation (%) correlates closely with lower damaged leaf area[17]. Late leaf spot resistant varieties are characterized by lower AUDPC and lower defoliation (%)[10,23]. Less number of late leaf spot resistant genotypes found in this study perhaps due to the limited number of genotypes evaluated. Using large number of evaluated genotypes, a higher number of late leaf spot resistant genotypes were identified by previous workers[10,24].

Leaf rust resistance of the genotypes evaluated in the present study was of low levels, where Kancil and Local Rote were 'susceptible' while Gajah, Bison and Jerapah were 'highly susceptible'. This result is in accordance with that of Inayati and Yusnawan[9], where the Indonesian released varieties Gajah, Kancil and Bison were also found to be 'highly susceptible' to leaf rust but the associated yield losses caused by the disease was not recorded. Late leaf spot and leaf rust resistance levels of Indonesian released varieties reported here were lower than those reported in their varietal descriptions, which ranged from 'moderately resistant' to 'resistant' reactions[12]. This might have been caused by changes in their resistance levels due to the evolving new pathogenic races of *C. personatum* and *P. arachidis*. Thus, this study results highlight the importance of regular evaluation and, hence improvement of late leaf spot and leaf rust resistance levels in the varieties that have been released for many years as their resistances may have been broken down by the newly arising races of the pathogens.

Late leaf spot and leaf rust diseases significantly affected pod yields as shown by the reduced pod yields under the diseased conditions. Reduced pod yields due to late leaf spot and leaf rust in the fields had been reported by other workers[3-5,10,25], but the percentages of yields losses of each tested respected genotype was not reported. The reduced yield of groundnut was due to leaf area damage and hence the decrease in photosynthetic leaf area resulting from necrotic spots, rust pustules and defoliation[9,26].

Late leaf spot-inducing pod yield loss reported here was higher than that reported by Utomo and Akin[27]. Higher percentages of yield loss caused by late leaf spot and leaf rust diseases were observed in previous studies by Subrahmanyam *et al.*[14] in the field (50-80%) and Inayati and Yusnawan[9] in the greenhouse (61-85%). Inayati and Yusnawan[9] reported that yield losses caused by late leaf spot and leaf rust in Indonesian released varieties Gajah, Bison and Kancil ranged from 65-70% in the greenhouse condition. Differences in yield losses in Indonesian released varieties reported here and that of the previous work[9,27] might have been caused by differences in environmental conditions and or the difference in pathogen races of *C. personatum* and *P. arachidis* employed.

Local Rote cultivar produced higher pod yields than Indonesian released varieties in both protected plots and diseased (late leaf spot and leaf rust) plots. This cultivar still produced significantly higher pod yield in the leaf rust plots despite its susceptible reaction to the disease. On the contrary, the released variety Kancil showed almost the same rust-susceptible reaction but produced the lowest pod yield. This implies that Local Rote was able to cope with the high leaf rust disease infection while still producing high yield, which may indicated its tolerance to the disease. Disease tolerant plants are those endure severe disease without severe losses in yield or quality or able to tolerate the presence the pathogen by suffering relatively little damage[28,29]. High yield performance of Local Rote in the current study during rainy season was consistent with that during dry season[11], indicating the genotypes' yield stability across seasons. Local Rote was also reported to be more tolerant to drought than the same Indonesian released varieties[11]. Thus, this study results provide evidence that local cultivar Local Rote is as superior as or even better than the released varieties in many traits and thus, it can be recommended for direct release and or used to develop groundnut varieties able to withstand late leaf spot and leaf rust diseases and produce high yield.

CONCLUSION

The present study results showed variation in resistance levels, yield performances and yield losses caused by late leaf spot and leaf rust diseases in the Indonesian groundnut genotypes. Local Rote was 'moderately resistant' while the Indonesian released varieties were 'susceptible' and 'highly susceptible' to late leaf spot. Kancil and Local Rote were 'susceptible' and other Indonesian released varieties were 'highly susceptible' to leaf rust. Pod yield loss caused by late leaf spot and leaf rust ranged from, respectively, 15-57% and 23-57%.

SIGNIFICANCE STATEMENT

The significant findings of this research is the available information on leaf spot and leaf rust resistance levels, yield performances and yield losses in Indonesian groundnut genotypes, which is of a great importance in control of groundnut foliar diseases. Local Rote cultivar was moderately resistant to leaf spot, fairly tolerant to leaf rust and high yielding in both diseased and no-disease conditions, thus it can be used to develop disease resistant and high yielding groundnut varieties. The Indonesian released varieties lost their resistance to both diseases and suffered high yield losses, which necessitates improvement of their resistance levels and more effective control strategies to be carried out. The results will advance the current knowledge in the field and help researchers, especially plant breeders and plant pathologist, to prevent high yield losses by developing resistant varieties and finding more efficient control measures for leaf spot and leaf rust diseases of groundnut.

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