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Evaluation of Leaf Spot Resistance and Agronomic Characteristics of Groundnut Advanced Breeding Lines

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Abstract

Leaf spot is a major groundnut disease in Indonesia and worldwide which has the potential to cause a yield loss of up to 60%. The use of leaf spot resistant cultivars is an easy, economical, and environmentally friendly way in groundnut cultivation to maintain high yields. The objective of the research was to evaluate the yields of 16 groundnut lines which had been developed to be leaf spot resistant and high yielding. The research was conducted in 2010 – 2011 at the Bogor Agricultural University (IPB) experimental station in Cikarawang, Indonesia. A randomized complete block design with three replications was used in the experiment. The groundnut lines evaluated were crosses between "Gajah", a cultivar which is known to be susceptible to leaf spot, and the resistant line GPNC- WS4. Four Indonesian cultivars "Gajah", "Jerapah", "Zebra" and "Sima" were used as control. The results showed that selected lines demonstrated better resistance to leaf spot compared to "Gajah". Their agronomic characteristics, such as pod number, filled pod number, pod weight, and kernel weight, were not significantly different from the control cultivars. The results of this study have provided information on groundnut leaf spot resistant lines for further evaluation.

Keywords: *Cercospora*, peanut, legumes, plant disease, high-yielding cultivar, Bogor

Introduction

Groundnut (*Arachis hypogaea* L.) is a second important legume crop after soybean in Indonesia. Groundnut originated from South America, but now has spread throughout the world particularly in the tropical and subtropical regions. Groundnut is an important source of fat and protein for human consumption as well as for animal feed and it contains high nutrition. The demand for groundnut production is high (Kasno, 2006) as the country continues to import amounting up to 200,000 tons per year (Balitan 2010). In Indonesia, groundnut harvest area had declined in the last five years from 620 563 ha to 454 349 ha (CBS 2015) and consequently had reduced yields from 84 to 77 million tons, whereas the productivity had risen from 1.16 ton per ha to 1.21 ton per ha (CBS, 2015). There is a great potential in the production of groundnut, however, there is low productivity by the farmers in Indonesia as compared to other sub-tropical regions (Baring et al, 2014). This may be due to the poor farming techniques employed, little access to disease resistant and high-yielding cultivars, poor seed quality, and diseases.

Leaf spot caused by *Cercospora arachidicola* and *Phaeoisariopsis personata* Berk.& Curtis is a severe fungal disease of groundnuts worldwide (Jackson, 2015). Emergence of circular brown or reddish-brown necrotic spots with yellow margins on older leaves is the initial symptoms shown and disease progress rapidly to other younger leaves in high humidity (Semangun, 1991). In extreme cases, leaves dry up killing the entire plant, hence reduces yields by up to 50% (Adisarwanto, 2001; Tsatsia and Jackson, 2016). Management of leaf spot widely implemented involves roguing of diseased or infected plants, shifting cultivation to prevent the buildup of primary inoculum in the fields and the use of resistant, early and high yielding cultivars.

The use of improved and high yielding cultivars is important for plant production. Ploidy difference between wild *Arachis* species and cultivated genotypes hinder transfer of useful alleles for important agronomical traits (Khera et al., 2014). Crop breeding is aimed at improving the crop's genetic potential that is adaptable to specific ecosystems, high yielding and has quality that meets the consumer tastes. High yielding cultivars that are supported with good production system will increase production and productivity.

The main aim of this study was to evaluate the yields of 16 selected groundnut lines previously developed by the Genetic and Plant Breeding Division of the Bogor Agricultural University in 1994. These lines were progenies of crosses between "Gajah", a local commercial cultivar and GPNC-WS4 line derived from interspecific cross between *Arachis hypogaea* (2n=4x=40, or tetraploids) with *Arachis cardenasii* (2n=2x=20, or diploids) and is known to be resistant to leafspot disease (Stalker and Beute, 1993).

Materials and Methods

The research was conducted in 2010 – 2011 at the IPB experimental station at Cikarawang in Bogor (-6.54; 106.73), West Java, Indonesia. Study site is located at an altitude of 250 m above sea level on latosols with daily average temperature of 32 to 36°C during the day. Chlorophyll analysis was performed at the Research Group for Crop Improvement (RGCI) Laboratory IPB, Darmaga, Bogor, Indonesia.

A total of 16 advanced generation groundnut lines known to be resistant to leaf spot disease were evaluated against "Gajah", "Jerapah", "Zebra" and "Sima" as the local checks.

Seeds were sown in 4x3 m^2 plots with a spacing of 40 cm between rows and 15 cm within rows. The experimental units were organized using the completely randomized block design with lines as the treatments and replicated three times. Urea at 50 kg per ha, SP-18 200 at kg per ha and KCl at 100 kg per ha were incorporated to the soil prior to planting. Insecticide containing active ingredient carbofuran with its recommended dosage rate was placed in each of the planting hole at planting. Agricultural lime at 500 kg per ha was applied into furrows at four weeks after planting to optimize pod filling. There was no fungicide used during the trial period and harvesting was done 100 days after planting (DAP).

Ten plants were randomly selected from one row of each plot of each cultivar and scored for plant height, number of branches at harvest, percentage length of the main stem with green leaves at harvest, dry harvest index, total filled and empty pods per plant, fresh weight of filled and empty pods per plant, seed weight per plant, weight of 100-dry seeds, and leaf chlorophyll content at eight weeks after planting (WAP). Percentage of green leaves was scored by measuring the length of the main stem (a) and the length of the main stem bearing green leaves (b) (usually at the upper part of the main stems); the percentage was calculated as (b/a) x 100%. Fresh stover weight was measured by weighing fresh shoots directly after harvest. Leaf chlorophyll was measured using the method by Wellburn (1994) and described in details in Yudiwanti et al. (2007).
Data was analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS) 9.4.software. Means were separated using the Dunnett test at p=0.05

Results and Discussion

During the duration of the study, the average rainfall recorded was 458.92 mm with a total of 21.2 days of rain. The temperature ranged from 25.1 to 26.7°C with an average of 25.8 °C. There was an increase in rainfall at 12 weeks after planting till the end of the cropping season which possibly may have affected the pod filling and ripening stage. The optimum temperature for groundnut growth in Indonesia ranges from 27 to 30°C (Adisarwanto et al., 1993), however, based on the result shown, the temperature was lower than the optimal hence may reduce photosynthesis rate which ultimately may affect growth and development of the crop (Sumarno and Slamet, 1993).

Groundnut showed no symptoms of leaf spot disease in their early growth that is from germination until about four weeks after planting. Leaf spot symptoms started to appear at five weeks after planting (WAP), showing small black to brown spots on the basal of the leaves. In severe cases, leaves dried up resulting in defoliation of older leaves, presumably due to higher humidity in the lower parts of the plants. Based on visual observations in the field, "Gajah" cultivar showed high severity of leaf spot and this was apparent by the low percentage of main branch bearing green leaves and lower fresh stover weight. However, less spots were observed on the more tolerant cultivars "Jerapah", "Zebra", and "Sima". Apart from leaf spot, other diseases observed included bacterial wilt (*Pseudomonas solanacearum*), rust (*Puccinia arachidis*), witchess broom (*Phytoplasma*), groundnut mottle (groundnut mottle virus / PeMoV), and groundnut stripe (Peanut Stripe Virus/PStV).

Agronomical Characteristics of Advanced Breeding Lines of Groundnut

The groundnut lines had different level of resistance to leaf spot disease and the criteria used to identify their characteristics include plant height, number of branches, chlorophyll content, number and weight of empty pods, and weight of 100-grains (Table 1).

Based on the result shown in Table 1, "Sima" was the tallest (79.1 cm) whereas GWS138A was the shortest (38.5 cm). The plant height of all other lines

Table 1. F-test recapitulation, mean, maximum, and minimum values of characters of advanced breeding lines

Characters	F-value	Mean	Maximum	Line	Minimum	Line
Plant height (cm)	5.78**	52.5	79.1	“Sima”	38.5	IPB-GWS138A
Branch number	5.98**	5.4	7.1	IPB-GWS79A	4.7	IPB-GWS39D
Length of main branch with green leaves (%)	1.71 ns	5.9	8.4	IPB-GWS74A	2.3	“Gajah”
Fresh stover weight per plant (g)	2.15 ns	14.9	19.6	IPB-GWS74A1	10.8	“Gajah”
Chlorophyll content (μmol/cm²)	2.70**	0.057	0.068	IPB-GWS27C	0.051	IPB-GWS110A2
Total pod number per plant	1.09 ns	9.7	12.3	IPB-GWS134D	7.3	IPB-GWS134A
Full pod number per plant	1.17 ns	9.4	11.8	IPB-GWS134D	6.6	IPB-GWS134A
Total pod weight per plant (g)	0.95 ns	10.6	13.3	IPB-GWS134D	7.3	IPB-GWS134A
Full pod weight per plant (g)	0.97 ns	10.5	13.2	IPB-GWS73D	7	IPB-GWS134A
Kernel weight per plant (g)	1.09 ns	7.2	9.8	IPB-GWS134A1	4.6	IPB-GWS134A
100 kernels weight (g)	20.5**	47.2	53.6	IPB-GWS138A	41.6	IPB-GWS110D
Yield index	1.11 ns	0.8	1.1	“Gajah”	0.5	IPB-GWS74A1

Note: * and ** show significantly different based on F-test at α 0.05 and 0.01, respectively.

was significantly lower than “Sima”, but it was not significantly different from “Gajah” as depicted in Table 2. Moreover, as per field observation, it was seen that the taller the plants the higher the tendency to easily lodge.

Lines IPB-GWS134D and IPB-GWS79A had one to two more branches than the control cultivars which having an average of five branches per plant. More branches means more leaves which will increase photosynthesis activity resulting in higher productivity. On the other hand, more branches per plant might reduce photosynthesis because of shading amongst leaves.

As shown in Table 3, “Gajah” and “Zebra” had a greater number of branches compared to other lines. GWS79A had the greatest number of branches per plant (7.1) whereas GWS39D had the least (4.7). More branches per plant will potentially result in more flowers and pod formation.

Leaf spot symptom was observed on the older leaves and progressed upwards, leaving only the younger leaves at the top. The percentage of green leaves in the plant indicates that those leaves were not infected (Kusumo, 1996). The proportion of green leaves to the total leaves could be one of the important indicators of plant’s resistance to leaf spot disease (Yudiwanti, 2007).

There were no significant differences amongst lines in the proportion of green branches per plant. GWS74A1 had the largest proportion of green branches (8.4%) compared to “Gajah”, which is known to be susceptible to leaf spot disease, yielding 2.3%.

Based on the chlorophyll content, GWS27C leaves had significantly more chlorophyll than “Gajah” and “Jerapah” but shown to be not significantly different to the leaf chlorophyll content of the other groundnut cultivars (Table 3). Chlorophyll is an organelle in plant cells that play a significant role in photosynthesis, and chlorophyll content is indicated by the green intensity of the leaves.

Furthermore, the lines tested had more pods per plant relative to the control cultivars although there was no significant difference amongst the different cultivars (Table 3). GWS134D lines had the highest number of pods (12.3) whereas GWS134A had the lowest (7.3). The number of pod per plant is affected by the success of flowering and growth of gynophores (Trustinah, 1993) and according to the result shown in this study, only about 55% of the flowers formed gynophores. Number of gynophores after flowering usually did not affect the pod production.

Seed formation begins after pods reach their maximum size that is between 52 to 57 days after planting or around three weeks after gynophore has penetrated the soil (Trustinah, 1993). Soil penetration by gynophores is necessary for pod development (Zharare et al., 1993) and this process is partially controlled by genetic factors (Gupta et al., 2016). Therefore, groundnut cultivars vary considerably in their pod-filling potentials. The Dunnett test results showed that there were no significant differences in the percentage of filled pods amongst all lines. Line that had the largest percentage of filled pods was IPB-GWS134D (11.8) and the lowest was IPB-GWS134A (6.6) (Table 3).

Table 2. Vegetative growth and level of resistance to leaf spot of advanced breeding lines

Line	Plant height	Branch number	Branch length with green leaves (%)	Chlorophyll content (μmol/cm²)	Fresh stover weight (g)
IPB- GWS18A1	56.4 c+d-	5.1	5.6	0.053	12.7
IPB-GWS27C	56.7 c+d-	5.4	5.6	0.068a+b+	15.1
IPB-GWS39B	43.3 d-	6.0	6.5	0.061	15.6
IPB-GWS39D	49.8 d-	4.7	4.3	0.062	11.8
IPB-GWS72A	51.9 d-	5.2	4.3	0.055	13.1
IPB-GWS73D	52.7 d-	5.4	6.4	0.055	18.7
IPB-GWS74A1	61.0 c+d-	5.2	8.4	0.053	19.6
IPB-GWS74D	56.2 c+d-	5.0	7.1	0.056	17.9
IPB-GWS79A	53.8 d-	7.1a+b+c+d+	6.1	0.052	18
IPB-GWS110A1	50.9 d-	4.8	7.3	0.054	14.7
IPB-GWS110A2	46.4 d-	5.5	6.7	0.051	12.6
IPB-GWS110D	54.4 d-	5.2	4.7	0.054	12.6
IPB-GWS134A	48.1 d-	5.0	6.9	0.062	12.3
IPB-GWS134A1	49.7 d-	5.8	5.5	0.055	13.3
IPB-GWS134D	61.0 c+d-	6.8a+b+c+d+	4.9	0.058	17.4
IPB-GWS138A	38.5 d-	4.8	5.5	0.056	13.1
“Gajah”	45.8	5.1	2.3	0.057	10.8
“Jerapah”	53.7	5.1	5.6	0.055	14.3
“Zebra”	39.9	5.0	7.4	0.060	16.9
“Sima”	79.1	5.1	6.2	0.059	17.7

Note: Values followed by a, b, c, d are significantly more (+) or less (-) from “Gajah”, “Jerapah”, “Zebra” and “Sima” , respectively, based on Dunnett’s test at α 0.05

Total pod weight of the tested lines was similar to those of the control cultivars (Table 3). IPB-GWS134D had the highest total pod weight per plant of 13.3 g, whereas IPB- GWS134A had the lowest (7.3 g). Pod weight is strongly influenced by environmental conditions during the phase of pod filling. Seed weight per plant for each line was not significantly different from the control cultivars. IPB-GWS134A1 had the highest seed weight (9.8 g) whereas IPB- GWS134A had the lowest (4.6 g). Seed weight contributes to crop productivity; however in this study the seed weight was lower than the groundnut yields, reported by Baring (2014). The low seed weight was most likely caused by the heavy rainfall towards the end of the growing season resulting in reduced light intensity and photosynthesis rate and subsequently low seed yields (Sumarno and Slamet 1993). Amongst the four control cultivars, “Zebra” had the largest seed weight per plant whereas the other lines had similar grain weight (Table 3).

Weight of 100 seed is a character that can affect the yield and as shown in Table 3, this character varied considerably amongst the lines. IPB-GWS138A had the greatest 100-seed weight (53.6 g) whereas IPB-GWS110D had the lowest (41.6 g). The performance of groundnut lines using this parameter was not significantly different to the local cultivars used as the control.

Stover weight indicates the efficiency of photosynthesis of the plants. There was no significant difference in the fresh stover weight between the selected lines and the control cultivars. IPB-GWS74A1 had the largest (19.6 g) whereas “Gajah” had the lowest (10.8 g) and this may be attributed to the severe defoliation caused by the leaf spot infection. However, “Gajah” yielded the highest harvest index of 1:13.

Best Groundnut Lines Selection

The total number of pods and number of pods indicate the genetic yield potentials of groundnut lines associated with leaf spot disease. Grain weight is more affected by environmental condition during pod filling stage Yudiwanti et al. (1998) and this may be due to pods formed prior to leaf spot infection

Table 3. Kernel characteristics of selected groundnut lines

Lines	Total pod per plant	Total filled pods	Total pod weight (g)	Filled pod weight (g)	Kernel weight (g)	100-kernel weight (g)	Dry yield index
IPB- GWS18A1	10.2	9.9	9.8	9.7	7.0	42.9	0.89
IPB-GWS27C	9.0	8.3	9.2	9.0	6.2	46.4	0.64
IPB-GWS39B	11.3	11.2	12.1	12.0	8.3	45.4	0.84
IPB-GWS39D	12.0	11.8	11.8	11.8	8.5	42.8	1.08
IPB-GWS72A	8.6	8.3	10.4	10.4	7.1	51.5	0.83
IPB-GWS73D	10.5	10.3	13.3	13.2	9.1	53.5	0.80
IPB-GWS74A1	8.7	8.3	8.6	8.5	5.4	49.5	0.46
IPB-GWS74D	8.0	7.8	8.9	8.9	5.9	50.3	0.55
IPB-GWS79A	11.7	10.9	12.0	11.8	8.1	50.3	0.71
IPB-GWS110A1	8.8	8.6	8.7	8.6	5.9	44.4	0.63
IPB-GWS110A2	11.7	11.4	11.0	10.9	8.1	44.7	0.99
IPB-GWS110D	10.2	10.1	10.0	10.0	6.9	41.6	0.88
IPB-GWS134A	7.3	6.6	7.3	7.0	4.6	44.4	0.57
IPB-GWS134A1	10.9	10.6	13.1	13.1	9.8	52.2	1.08
IPB-GWS134D	12.3	11.8	13.3	13.2	9.3	46.8	0.86
IPB-GWS138A	7.7	7.5	9.3	9.2	5.9	53.6 b+	0.86
“Gajah”	10.0	9.5	10.4	10.2	7.1	43.9	1.13
“Jerapah”	7.9	7.6	9.8	9.7	6.2	52.4	0.70
“Zebra”	9.9	9.4	12.6	12.4	8.8	41.7	0.77
“Sima”	8.1	7.8	10.9	10.8	7.2	45.9	0.64

Note: Values with a, b, c, and d show significantly more (+) or less (-) than “Gajah”, “Jerapah”, “Zebra” and “Sima” respectively based on Dunnett test at α 0.05

Table 4. Total number of pods and filled pods per plant of the groundnut lines

Selected Lines	Total number of pods per plant	Total number of filled pods per plant
IPB-GWS134D	12.3	11.8
IPB-GWS39D	12.0	11.8
IPB-GWS79A	11.7	10.9
IPB-GWS110A2	11.7	11.4
IPB-GWS39B	11.3	11.2
IPB-GWS134A1	10.9	10.6
IPB-GWS73D	10.5	10.3
IPB-GWS110D	10.2	10.1
IPB-GWS18A1	10.2	9.9
“Gajah”	10.0	9.5
“Zebra”	9.9	9.4
IPB-GWS27C	9.0	8.3
IPB-GWS110A1	8.8	8.6
IPB-GWS74A1	8.7	8.3
IPB-GWS72A	8.6	8.3
“Sima”	8.1	7.8
IPB-GWS74D	8.0	7.8
“Jerapah”	7.9	7.6
IPB-GWS138A	7.7	7.5
IPB-GWS134A	7.3	6.6

Nine lines that is, IPB-GWS134D, IPB-GWS39D, IPB-GWS79A, IPB- GWS110A2, IPB-GWS39B, IPB-GWS134A1, IPB-GWS73D, IPB-GWS110D, and IPB-GWS18A1, had higher number of pods per plant than the control cultivars (Table 4).

Conclusion

Nine groundnut lines in this study, IPB-GWS134D, IPB-GWS39D, IPB- GWS79A, IPB-GWS110A2, IPB-GWS39B, IPB-GWS134A1, IPB-GWS73D, IPB-GWS110D, and IPB-GWS18A1, demonstrated better resistance to leaf spot when compared to the commercially grown cultivar "Gajah" (control cultivar) as indicated by their growth and yield parameters. These nine advanced breeding lines had more than ten pods per plant and their growth was not significantly different to the local cultivars used as the control. Information derived from this study will be useful for further research into the development of leaf spot resistant groundnut cultivars for commercialization.

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