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Screening of elite black soybean lines for resistance to rust disease, *Phakopsora pachyrhizi*

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Abstract. *Krisnawati A, Gatut-Wahyu AS, Adie MM. 2016. Screening of elite black soybean lines for resistance to rust disease, Phakopsora pachyrhizi. Biodiversitas 17: 134-139.* Indonesian tropical climate is ideal for both of soybean growth and harmful disease development. Soybean rust, *Phakopsora pachyrhizi*, has been a serious disease in Indonesia and may have an impact on soybean production. The objective of the study was to evaluate the resistance of elite black soybean lines to rust disease. Agronomic characters (yield and yield component) were evaluated based on research conducted in 16 soybean production centers in Indonesia. The evaluation for rust resistance was conducted in Indonesian Legumes and Tuber Crops Research Institutes' greenhouse in 2011 using ten elite black soybean lines. The seed yield of ten black soybean lines grown in 16 locations ranged from 2.51-2.88 t/ha, with an average of 2.59 t/ha. The eight lines showed higher yield than check cultivar of Malika, whereas only one line (W9837 × Cikuray-66) had higher yield than check cultivar of Detam-1. Resistance reaction to rust disease fluctuates over time. Two elite lines of Cikuray × W9837-171 and Cikuray × W9837-184 showed a consistent resistance to *P. pachyrhizi*. W9837 × Cikuray-66 and check cultivar of Detam-1 consistently showed a moderately resistant. In the terms of agronomic aspects, W9837 × Cikuray-66, which has moderately resistant to *P. pachyrhizi*, produced the highest yield (2.88 t/ha). The both of resistant lines of Cikuray × W9837-171 and Cikuray × W9837-184 have early maturity, and medium seed size with yield 2.57 t/ha and 2.56 t/ha, respectively. The resistant and moderate lines with high yield, early maturing, and medium seed size found in this study playing an important role in the soybean varietal development program for rust resistance in Indonesia.

Keywords: Black soybean, *Glycine max*, resistance, rust disease, screening, yield

INTRODUCTION

Soybean rust, caused by *Phakopsora pachyrhizi*, has been a major disease limiting soybean production and has caused significant economic annual yield losses in Asia, Africa, and South America (Hartman et al. 2004; Levi 2004; Panthee et al. 2007; Jarvie 2009; Khanh et al. 2013). Soybean rust also has become one of the obstacles to increase soybean production in Indonesia due to the tropical climate (high temperature and humidity) providing suitable conditions for disease development, especially during the dry season.

Soybean rust becomes the most destructive foliar disease of soybean worldwide due to the widespread distribution and the potential for severe yield losses (Hartman et al. 2005). Soybean rust symptoms generally occur first on the leaves at the base of the plant and spread up to the canopy as the disease severity increases. Rust symptoms include presence of tan to dark brown or reddish brown lesions (Sinclair and Hartman 1999). An increase in leaf density will result in leaf yellowing, early leaf senescence, and yield losses (Tichagwa 2004). The heavy defoliation due to rust disease was affected the pod formation and pod filling (Yang et al. 1991).

Yield losses due to soybean rust can occur 100% depending on the weather conditions and degree of plant susceptibility (Kawuki et al. 2003a; Mueller et al. 2009). In southern Japan, yield losses were estimated at 15-40% (Bromfield 1976), whereas the yield losses 10-30% was common in Southern China and can be over 50% in severe epidemics (Bromfield et al. 1980). In Korea, yield losses in susceptible and tolerant cultivar were 68.7% and 22.3%, respectively (Shin and Tschanz 1986). In Indonesia, soybean rust has potential to reduce the soybean yield from 10% up to 90% (Sumartini 2010).

Resistance to soybean rust is manifested phenotypically by red-brown lesions and characterized by the three plant responses that have been shown to be associated with single dominant genes for soybean rust resistance, i.e. an immune response, reddish-brown lesions (or incomplete resistance), and the susceptible tan lesions (Bromfield, 1984). Six major resistance genes (Rpp1, Rpp2, Rpp3, Rpp4, Rpp5, and RppHyuuga) controlled the resistance to soybean rust have been identified with different resistance to a limited set of rust isolates (Hartman et al. 2005; Maphosa et al. 2012). Several strategies for controlling soybean rust have been applied, such as fungicide application, cultural and seed sanitation technique, and the use of resistant cultivar. The use of adapted soybean cultivars with resistance to *P. pachyrhizi* is cost-efficient ways and harmless to the environment (Goellner et al. 2009; Hassan et al. 2014; Yamanaka et al. 2011).

Singh and Schwartz (2011) stated that there are principal factors that play important role in the implementation of strategies and methods used for breeding for resistance to soybean rust that consist of: (i)

the genetic distance between the cultivar to be improved and resistant donor germplasm, (ii) the availability of direct and indirect screening methods, (iii) the genetics of resistance, and (iv) the number of resistances and other traits to be improved. Furthermore, the choice of the selection method will depend on various factors including the breeding objective, genetic variability, available facility and infrastructures, and the personal skill of breeding team (Vishnyakova and Seferova 2013).

In Indonesia, the major emphasis in soybean varietal improvement (soybean breeding) is focused on producing high-yielding cultivar. Within 97 years (1918-2015), the Indonesian government has successfully released nine black soybean varieties (ILETRI 2014). The potential development of soybean as a raw material for industry is reflected in the last ten years, showed by increasing demands for black soybean, thus contributes to farmers' income. Black soybean in Indonesia is used as raw material for soy sauce industry, both in the domestic and large scale industries. Since soybean rust becomes serious disease in Indonesia, it is important to develop black soybean genotypes with high yield potential and less yield loss from soybean rust.

The objective of the study was to evaluate the resistance of elite black soybean lines to rust disease, *Phakopsora pachyrhizi*.

MATERIALS AND METHODS

The genotypes used in this study were black soybean lines derived from the crossing of parental lines W9837, MLG 3102, Cikuray (black soybean); and 100H (yellow seed coat). The research materials consist of eight black soybean promising lines (Cikuray × W9837-171, Cikuray × W9837-105, W9837 × Cikuray-66, W9837 × 100H-236, MLG 3102 x Cikuray-435, Cikuray x W9837-181, Cikuray x W9837-184, and W9837 × Cikuray-26), and two commercial check varieties (Detam-1 and Malika).

The yield trial was conducted in 16 soybean production centers in 2011 cropping season. A randomized complete block design with four replicates was arranged in each location. Each line was planted on 2.8 m × 4.5 m plot size, 40 cm × 15 cm plant distance, two plants per hill. Fertilizer of 50 kg/ha Urea, 100 kg/ha SP36 and 75 kg/ha KCl were applied before sowing time. Weed, insect and disease were controlled intensively. The parameters measured consists of yield and yield components: days to flowering (when 50% of the plant population have been flowering), days to maturity (calculated if 95% of the leaves have turned yellow), plant height (taken from average of 5 randomly sample plants in cm), 100-seeds weight (determined by randomly weighing 100 seeds in gram), and seed yield (randomly taken from the seed yield per plot and converted to t/ha). All the data collected were subjected to statistical analysis of variance (ANOVA) of mean performance of genotypes, locations, and their interaction. Least significant differences (LSDs) were determined at 5% probability level.

Evaluation of rust resistance was conducted in Indonesian Legumes and Tuber Crops Research Institutes (ILETRI)'s screen house in Malang, East Java, Indonesia in dry season 2011 using a completely randomized design. The first factor was inoculation treatment (without inoculation and with inoculation), and the second factor was 10 black soybean genotypes. Each genotype was planted in a plastic polybag (Φ =15 cm), two plants per polybag. At 30 days after planting, plants were inoculated with uredospore. The inoculation density was 10,000 spores per millimeter. Spore suspension was originated from naturally infected plants. Uredospore were harvested from the lower surface of infected leaves. Rust severity was determined at weekly intervals (38, 45 52, 69, and 76 days after planting) using IWGSR (International Working Group on Soybean Rust) rating system (Shanmugasundaram 1977).

The IWGSR rating system uses a three-digit rating score to record the rust severity (Table 1). The first digit denotes the upper bond position of most diseased leaves in the canopy of the plant. The second digit denotes the density of rust lesions on most of the diseased leaves. The third digit denotes the infection type on most of the diseased leaves. The rating scale of each digit is explained as Table 1, and relationship between disease reactions and IWGSR ratings for soybean rust is explained as Table 2 (Yang 1977).

Table 2. Relationship between disease reactions and IWGSR ratings for soybean rust

Disease reaction	IWGSR rating
Immune	111
Resistance	122, 123, 132, 133, 222, 223
Moderately resistant	142, 143, 232, 233, 242, 243, 322, 323
Moderately susceptible	332, 333
Susceptible	343

Table 1. The International Working Group on Soybean Rust (IWGSR) rating system

Parameter criteria	Score	Description
First digit: upper bond position of most diseased leaves	1	1 = bottom third of the leaf canopy of the plant
	2	2 = middle third of the leaf canopy of the plant
	3	3 = upper third of the leaf canopy of the plant
Second digit: density of rust lesions on most diseased leaves	1	1 = no lesion
	2	2 = light lesion density (<1-8 lesions per cm^2)
	3	3 = medium lesion density (9-16 lesions per cm^2)
	4	4 = heavy lesion density (>16 lesions per cm^2)
Third digit: infection type on most diseased leaves	1	1 = no pustules
	2	2 = non-sporulating pustules
	3	3 = sporulating pustules

RESULTS AND DISCUSSION

Yield and yield component

The combined analysis of variance was presented in Table 3. Genotype, location, and genotype by environment interaction were highly significant. The agronomic characters of ten black soybean lines in 16 environments are presented in Table 4. All the data of agronomic characters (yield and yield components) were of the average of 16 locations. The seed yield of ten black soybean lines grown in 16 locations ranged from 2.51-2.88 t/ha, with an average of 2.59 t/ha. The eight black soybean lines showed higher yield than check cultivar of Malika, whereas only one line (W9837 × Cikuray-66) had higher yield than check cultivar of Detam-1.

The yield component consists of days to flowering, days to maturity, plant height, seed weight, and seed yield. Days to flowering ranged from 33 to 35 days, days to maturity ranged from 74 to 82 days, plant height varied from 51.16 to 63.46 cm, seed weight ranged from 10.92 to 13.60 g/100 seeds, and seed yield ranged from 2.46 to 2.88 t/ha. In Indonesia, soybean maturing day classified as late maturity (> 90 days), medium maturity (80-90 days), and early maturity (<80 days). According to maturity classification, all elite lines showed early maturing day, except the check cultivars which showed medium maturity (Table 4). The earliest maturity (under 76 days) were mostly showed by lines derived from the crossing of Cikuray × W9837, i.e. Cikuray × W9837-171 (74 days), Cikuray × W9837-105 (75 days), Cikuray × W9837-184 (75 days), and Cikuray × W9837-181 (75 days).

The soybean seed size are categories into three: small (< 10 g/100 seeds), medium (10-14 g/100 seeds), and large size (> 14 g/100 seeds (PVT 2007). The seed weight of all lines was categorized as medium-seeded size (Table 4). The medium soybean seed size in Indonesia is desirable for tofu industry.

Table 3. The combined analysis of variance for black soybean yield in 16 locations. 2011.

Source	df	Sum of Square	Mean of Square
Location (L)	15	12.6600	0.8440**
Genotype (G)	9	7.5400	0.8380**
G × L	135	23.3800	0.1730**
Error	432	44.0700	0.1020
CV (%)	12.31		

Note: CV = coefficient of variation; ** = significant at p = 0.01

Table 4. Agronomic characters of 10 black soybean elite lines in 16 locations, 2011

Black soybean lines	Days to flowering	Days to maturity	Plant height (cm)	Seed weight (g)	Yield (t/ha)
Cikuray × W9837-171	34 d	74 g	57.55 b	11.22 de	2.57
Cikuray × W9837-105	34 e	75 f	58.28 b	10.98 ef	2.58
W9837 × Cikuray-66	34 e	75 ef	56.87 bc	11.75 c	2.88
W9837 × 100H-236	36 a	76 de	53.16 d	11.01 ef	2.54
MLG 3102 × Cikuray-435	33 f	76 cd	55.41 c	11.36 d	2.54
Cikuray × W9837-181	35 c	75 ef	57.49 b	10.92 f	2.64
Cikuray × W9837-184	35 cd	75 f	55.04 c	10.96 ef	2.56
W9837 × Cikuray-26	33 f	76 c	51.16 e	12.20 b	2.51
Detam-1	34 e	82 a	57.36 b	13.60 a	2.66
Malika	35 b	81 b	63.46 a	11.32 d	2.46
Average	34	77	56.58	11.53	2.59
LSD (5%)	0.31	0.41	1.89	0.32	0.96

Note: Value within the same column followed by the same letter are not significantly different at the 0.05 level according to LSD test

Table 5. Rust rating score of ten elite black soybean lines at five weekly intervals, 2014

Black soybean lines	Rating score according to IWGSR														
	38 DAP			45 DAP			52 DAP			69 DAP			76 DAP		
Cikuray × W9837-171	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2
Cikuray × W9837-105	2	3	2	2	3	2	2	3	3	2	3	3	2	2	3
W9837 × Cikuray-66	2	2	2	2	2	2	2	3	2	2	3	3	2	3	3
W9837 × 100H-236	2	2	2	2	2	2	2	2	2	2	3	2	2	3	2
MLG 3102 × Cikuray-435	2	2	3	2	2	3	2	3	3	2	3	2	3	3	3
Cikuray × W9837-181	1	2	2	2	3	2	2	3	2	2	2	2	2	2	2
Cikuray × W9837-184	2	2	2	1	2	2	1	3	2	1	2	2	2	2	2
W9837 × Cikuray-26	2	3	3	2	2	3	2	2	3	2	2	2	2	3	2
Detam-1	2	2	2	2	3	3	2	3	3	2	3	3	2	3	3
Malika	2	2	2	2	3	3	3	3	3	3	3	3	3	4	3

Note: DAP = days after planting, IWGSR = International Working Group on Soybean Rust (Yang 1977)

Table 6. Black soybean resistance to rust at five weekly intervals, 2014

Black soybean lines	Resistance criteria according to IWGSR				
	38 DAP	45 DAP	52 DAP	69 DAP	76 DAP
Cikuray × W9837-171	R	R	R	R	R
Cikuray × W9837-105	MR	MR	MR	MR	R
W9837 × Cikuray-66	R	R	MR	MR	MR
W9837 × 100H-236	R	R	R	MR	MR
MLG3102×Cikuray-435	R	R	MR	MR	MS
Cikuray × W9837-181	R	MR	MR	R	R
Cikuray × W9837-184	R	R	R	R	R
W9837 × Cikuray-26	MR	R	R	R	MR
Detam-1	R	MR	MR	MR	MR
Malika	R	MR	MS	MS	S

Note: DAP = days after planting, IWGSR = International Working Group on Soybean Rust (Yang 1977). R = resistant, MR = moderately resistant, MS = moderately susceptible

The rust resistance evaluation

The rust severity of ten black soybean lines was determined based on rating score, and evaluated at weekly intervals, consist of 38, 45 52, 69, and 76 days after planting (Table 5). According to the resistance criteria (Yang 1977), the rust resistance of black soybean elite lines consists of resistant (R), moderately resistant (MR), and moderately susceptible (MS) (Table 6).

At 38 days after planting (DAP), most soybean lines showed resistant reactions to the disease. At 45 DAP, which plants in the pods formation, the number of resistant lines decreased. The next intervals of evaluation at 52 DAP (pod filling stage/R5), 69 DAP (full pod filling stage/R5-R6), and 76 DAP (full seed/R6) showed a gradually decreasing resistance.

Discussion

Black soybeans play important roles in the industrial sector in Indonesia. Besides used as raw material for producing soy sauce, soybean with black seed coat are known to have high levels of phenolic and anthocyanin, and also have antioxidant and anti-inflammatory effects (Ku et al. 2000; Astadi et al. 2009; Xu et al. 2012). The main focus of the new soybean cultivar development in Indonesia involves breeding for high potential yield with desired characteristics, such as high nutritional contents and tolerance or resistance to biotic and abiotic stresses.

Seed yield as a complex trait consists of components which serve as significant indicator for the seed yield of soybean. The combined analysis of variance indicated that treatments (genotype, location, and genotype by environment interaction) were highly significant, showing that genotypes responded differently to the environments used during the study.

The yield and yield component showed variability between locations. The check cultivar of Detam-1 (2.66 t/ha) had higher yield than Malika (2.46 t/ha). Detam-1 is Indonesian black soybean varieties with high protein content (up to 45.36%), and released in 2008. Malika released as black soybean variety in 2007. The elite line of W9837 × Cikuray-66 produced the highest yield (2.88 t/ha); it is higher than the two check cultivars of Detam-1 and Malika (2.66 t/ha and 2.46 t/ha, respectively). In addition to high yield, this line also has characteristics of early maturity and medium seed size.

Indonesia as the tropical country receives plentiful sunshine throughout the year. This is an ideal environment for both of growing soybeans and the development of soybean pests and disease. In addition, the soybean cultivation mostly in the dry season II (June-September), which the pest and disease incidences occurred in the highest frequency.

The resistance evaluation to rust disease showed variation between weekly intervals. Most of the soybean lines showed resistant reactions at 38 days after planting, except the two lines (Cikuray × W9837-105 and W9837 × Cikuray-26) which were moderately resistant. In Indonesia, the soybeans plant age at 38 DAP were mostly in the first reproductive (R1) growth stage (Adie and Krisnawati 2007). The R1 growth stage is beginning flowering (Fehr and Caviness 1977). In the pods formation stage (45 DAP), the number of resistant lines were decreased. A total of six lines showed resistance reaction, and the rest were moderately resistant. Furthermore, the next intervals of the evaluation showed a gradually decreasing resistance. There were four resistant elite lines for each week interval. According to Kumudini et al. (2008), this is reasonable since disease severity increased as reproductive development advanced. Ribeiro et al. (2007) also stated that during the pod filling stage (R5-R6) become the most critical time due to the greater increase of the disease attack and causing the significant yield losses. Furthermore, it has been reported that soybean yield is most sensitive to leaf defoliation injury at the R5 growth stage (Fehr and Caviness 1977; Fehr et al. 1981). The overall results showed that Cikuray × W9837-171 and Cikuray × W9837-184 showed a consistent resistance to *P. pachyrhizi*, whereas the check cultivar of Detam-1 and W9837 × Cikuray-66 showed a consistent moderate resistance at 52 DAPS, 69 DAP, and 76 DAP. The other check cultivar of Malika showed a moderately susceptible reaction in pod filling formation, and then showed decreasing resistance to susceptible in full seed/R6.

A key requirement in breeding efforts is the screening of plants for resistance to diverse pathogen populations to identify cultivars that are likely to withstand variable pathogen populations (Harman et al. 2005). Several screening trials have been conducted for identification of resistant or tolerance genotypes. In Brazil, Santa Rosa, FT-1 and Uniao were identified as resistant cultivars and all the varieties and germplasm from US were found to be susceptible during screening trials (Ribeiro et al. 1985). Kawuki et al. (2003b) screened soybean germplasm for rust resistance at the NaCRRI and found none of the screened materials to be immune to rust infection. A total of 8 accessions were resistant, 45 moderately susceptible, 31 susceptible and 112 very susceptible. Screening through artificial inoculation using segregating populations (F3) of two crosses involving two high yielding varieties JS335 and JS9305 (both susceptible to rust), and one germplasm

line EC241780 (resistant to rust) were conducted in India. Six among the 62 progeny lines showed resistant or moderately resistant to rust (Shivakumar et al. 2011). Furthermore, screening trial against soybean rust under natural epiphytotic conditions at Barapani (Meghalaya, India) revealed that only two lines NRC 80 and MAUS 417 were moderately susceptible. There were no line or variety was in the moderately resistant or resistant category (Baiswar et al. 2012). The newest study in ten Indonesian soybean lines resulted that there were no line identified as resistant line (Sumartini and Kuswantoro 2014).

A high yielding varieties with rust resistance is one of pursued goal in Indonesian soybean breeding program. The both of resistant black soybean elite lines of Cikuray × W9837-171 and Cikuray × W9837-184 have agronomic characteristics of early maturity and medium seed size. These lines produced yield 2.57 t/ha and 2.56 t/ha, respectively; higher than check cultivar of Malika. The highest yield was produced by W9837 × Cikuray-66 (2.88 t/ha), which has moderately resistant to *P. pachyrhizi*. The resistant and moderate lines found in this study can be used as a source in breeding programs and/or can be developed to be a new black soybean cultivar with desirable characteristics.

To conclude, resistance reaction of black soybean elite lines to rust disease fluctuates over time. Two elite lines of Cikuray × W9837-171 and Cikuray × W9837-184 showed a consistent resistance to *P. pachyrhizi*, and two others (W9837 × Cikuray-66 and check cultivar of Detam-1) consistently showed a moderately resistant, respectively. Resistant and moderately resistant soybean lines with early maturity and medium seed size identified in this research may provide the gene source needed for future development of soybean cultivars with soybean rust resistance in Indonesia.

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