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Evaluation of soybean genotypes for resistance to rust disease (*Phakopsora pachyrhizi*)

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Abstract. *Sulistyo A, Sumartini. 2016. Evaluation of soybean genotypes for resistance to rust disease (*Phakopsora pachyrhizi*). Biodiversitas 17: 124-128.* Pest and disease are one of limiting factor in soybean cultivation in Indonesia. One of the diseases that can reduce soybean production is rust diseases caused by *Phakopsora pachyrhizi*. The use of resistant varieties can reduce yield losses due to this disease. The aim of this study was to evaluate the resistance of soybean genotypes to rust disease and to study the interaction between agronomic traits with rust disease on soybean genotypes. This study was conducted at a screen house of the Indonesian Legumes and Tuber Crops Research Institute (ILETRI) in Malang, Indonesia from April to July 2015. A total of 10 soybean genotypes consist of eight lines and two varieties (Argomulyo and Grobogan) was evaluated for rust resistance to rust disease. The eight lines tested were a progeny of a cross between offspring of IAC 100 (resistant to rust disease) with high yielding soybean varieties (Argomulyo and Grobogan). The experiment was arranged as randomized completely block design with four replications. Each genotype planted in five plastic pots for each replicate. Three weeks after planting, all plants were inoculated with rust disease. Inoculation was done by spraying a suspension of spores (spore density of 10^4 spore mL^{-1}) to the surface of leaves. Observations were carried out on rust disease severity based on the method of International Working Group on Soybean Rust rating system, days to flowering, plant height, number of branches, number of fertile nodes, number of pods, and seed weight per plant. The results showed that all genotypes classified as moderately resistant to rust disease. In this study, days to flowering and plant height influence the development of rust disease severity. There are three lines that have seed weight per plant significantly heavier than Argomulyo (4.97 g) and Grobogan (4.30 g), namely K/I100//B63///G-7 (6.55 g), K/I100//B63///G-8 (6.15 g), and I100/B54//A-5 (5.85 g). The high value of the scales of seed weight per plant for the three lines is supported by high-performance plants with a lot of number of fertile nodes and pods. These three soybean genotypes potentially serve as genetic material to develop high yielding soybean varieties and resistant to rust disease.

Key words: disease severity, *Glycine max*, IWGSR, selection, soybean lines
Abbreviations: DAP (Days after planting), PDL (Position of diseased leaves), DL (Density of lesions/cm^2), DE (Disease existence), TI (Type of infection), RC (Reaction criteria), R (Resistance), MR (Moderately resistant)

INTRODUCTION

Soybean is one of the staple food commodities and a major source of vegetable protein in Indonesia. These commodities have a strategic value which ranks third after rice and maize because every day it is consumed by most people in Indonesia with an average consumption rate of 8.12 kg per year (Sudaryanto and Swastika 2007). Domestic soybean demand will continue to increase in line with the rise of Indonesia population. However, domestic soybean productions are only able to fulfill a small portion (35%) of the needs of soybean, and the remaining is obtained through imports (Direktorat Akabi 2013). The dependence of Indonesia on soybean import will be a serious threat on food security in the future (Supadi 2009). One solution to overcome this problem is to increase domestic soybean production.

One of the obstacles to increase soybean production in Indonesia is an infection of rust disease caused by the fungus, *Phakopsora pachyrhizi*. Yield losses due to this disease range from 28% in Argentina (Formento 2008) and 75% in Brazil (Yorinori et al. 2005). In Indonesia, the rust diseases found in soybean production centers in Sumatra, Java, Bali, West Nusa Tenggara, Kalimantan and Sulawesi (Semangun 2008). According to Huang and Wu (2009), there is no single method that is capable of controlling crop diseases satisfactory, but a combination of effective control measures may enhance protection of crops from diseases as well as reduce production inputs for crops. Sumartini (2010) states that environmentally-friendly control methods of rust disease include planting a soybean resistant variety, application of botanical fungicide made of oil clove, and use of antagonistic bacteria (*Bacillus*) as well as antagonistic fungi (*Verticillium*).

Most soybean crop improvement programs seek to develop rust resistant and high yielding varieties. The first step that must be done is to get the source of resistance genes. According to Tukamuhabwa and Maphosa (2010), recently there are six race-specific genes have been identified, namely Rpp1, Rpp2, Rpp3, Rpp4, Rpp5 and Rpp? (Hyuuga). In addition, there is also recessive gene have been reported to participate in controlling on soybean resistance to rust disease (Calvo et al. 2008).

Research on evaluation of soybean genotypes for resistance to rust disease has been carried out in several programs. One of the activities of screening germplasm in large quantities has been done in the USA by Miles et al. (2006). More than 16,000 soybean accessions of the USDA germplasm collection have been evaluated for resistance to soybean rust disease and acquired approximately 805 accessions that showing a low mean visual severity or the presence of a red-brown reaction. Other researchers have also reported the results of screening of its genetic material in Vietnam (Pham et al. 2009; Pham et al. 2010) and Nigeria (Twizeyimana et al. 2008). The aim of this study was to evaluate the resistance of soybean genotypes to rust disease and to study the interaction between agronomic traits with rust disease on soybean genotypes.

MATERIALS AND METHODS

Plant material and experimental design

A total of ten soybean genotypes, consisting of eight lines and two varieties (Argomulyo and Grobogan) were tested for resistance to rust disease. The eight lines tested were obtained from the cross between the descendants of IAC 100 with high yielding soybean varieties (Argomulyo and Grobogan). Martins and Juliatti (2014) found that IAC 100 has a partial resistance to rust disease. The experiment was conducted at a screen house of Indonesian Legumes and Tuber Crops Research Institute (ILETRI) in Malang, Indonesia from April to July 2015. Each genotype were planted in 20 plastic pots and laid out as a randomized completely block design with four replications, each replicate consist of five plastic pots.

Preparation of spore suspension and inoculation of rust disease

Spore suspension was prepared in the laboratory one day before inoculation. Spore density used was 10^4 mL^{-1}. Infected plants naturally in the field were used as a source of inoculums. Infected leaves were taken to the lab to be incubated at 100% humidity condition for 24 hours. Afterward, the spores were taken using a brush and diluting with distilled water. Furthermore, spore suspension was homogenized by using Tween-20 two drops per liter. Spore suspension obtained was used for inoculation of all the genotypes tested. Inoculation was done on a three-week-old plant by spraying the spore suspension to the surface of the leaves. At three-week-old plant, soybeans have formed 3-4 trifoliolate leaf. According to Pham et al. (2009), the inoculation may be applied at the second or third trifoliolate leaf. Additionally, inoculating on a three-week-old plants aim to ensure that the rust disease symptoms can appear before the plant flowering (R2 phase).

Observations and data analysis

Observations were carried out on rust disease severity, days to flowering, plant height, number of branches, number of fertile nodes, number of pods, and seed weight per plant. The observation of rust disease severity based on the method of International Working Group on Soybean Rust (IWGSR) rating system and carried out at two and three weeks after inoculation. According to Shanmugasundaram (1977) this method uses a system of the three-digit score to categorize soybean resistance against rust disease. The first digit indicates the top position of diseased leaves (1 = bottom third of the canopy, 2 = middle third of the canopy, 3 = upper third of the canopy). The second digit indicates the density of rust lesions on the most diseased leaves (1 = no lesions, 2 = 1-8 lesions/cm^2, 3 = 9-16 lesions/cm^2, 4 = more than 16 lesions/cm^2). The third digit indicates the type of infection (1 = no pustule, 2 = no spores in pustules, 3 = pustules with spore). The data obtained was statistically analyzed using PKBT-STAT 1.0 software, except for rust disease severity. The category rust disease resistance based on a three-digit scoring system shown in Table 1.

RESULTS AND DISCUSSION

Rust disease severity

The results showed that the incubation period of rust disease (time from inoculation until the appearance of disease symptoms) was between 7 to 14 days for all the genotypes tested (data not shown). The reaction of soybean genotypes to rust disease on the first observation showed that all the tested soybean genotypes classified as resistant to rust diseases (Table 2). In the following week, there was an increase reaction to the rust disease that causes a change in resistance reaction of all soybean genotypes. In the second assessment all soybean genotypes categorized as moderately resistant to rust disease (Table 2).

Yield components

Analysis of variance showed that there were significant differences almost for all characters were observed, except number of branches which were not significantly different among genotypes tested (Table 3). The varieties of Argomulyo and Grobogan categorized as early flowering genotypes, 33 days after planting (DAP) for each variety. There was only one soybean line among eight lines which has days to flowering similar to both varieties, namely K/I100//B63///G-7. Two soybean lines, namely K/I100//B63///G-8 and I100/B54//A-5 has the highest performance, with a plant height of each line were 49.50 and 48.25 cm, respectively. The soybean line I100/B54//A-5 also has a lot of number of fertile nodes (16.00), followed by I100/K8//A-1 with the number of fertile nodes as much as 15.50.

Table 1. 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 2. Disease reactions and resistance criteria of 10 soybean genotypes to rust diseases

Genotypes	First observation						Second observation					
	PDL	DL	DE	TI	Score	RC	PDL	DL	DE	TI	Score	RC
I100/K8//A-1	1	10	3	2	132	R	2	15	3	3	233	MR
I100/K8//A-2	1	7	2	2	122	R	2	11	3	3	233	MR
I100/K14//G-3	1	10	3	2	132	R	2	12	3	3	233	MR
I100/K14//G-4	1	10	3	2	132	R	2	11	3	3	233	MR
I100/B54//A-5	1	9	3	2	132	R	2	11	3	3	233	MR
I100/B54//A-6	1	7	2	2	122	R	2	11	3	3	233	MR
K/I100//B63///G-7	1	7	2	2	122	R	2	14	3	3	233	MR
K/I100//B63///G-8	1	6	2	2	122	R	2	10	3	3	233	MR
Argomulyo	1	11	3	2	132	R	2	12	3	3	233	MR
Grobogan	1	10	3	2	132	R	2	12	3	3	233	MR

Note: PDL = Position of diseased leaves, DL = Density of lesions/cm^2, DE = Disease existence, TI = Type of infection, RC = Reaction criteria, R = Resistance, MR = Moderately Resistant

Table 3. Yield and its components of 10 soybean genotypes in rust disease inoculated conditions at screen house

Genotypes	Days to flowering (DAP)	Plant height (cm)	Number of branches	Number of fertile nodes	Number of pods	Seed weight per plant (g)
I100/K8//A-1	38.00^{a}	44.50^{abcd}	2.75	15.50^{ab}	33.00^{a}	4.70^{cd}
I100/K8//A-2	38.50^{a}	42.75^{bcde}	2.00	12.00^{cde}	23.50^{bc}	3.88^{d}
I100/K14//G-3	34.75^{b}	39.00^{de}	1.75	11.25^{de}	25.00^{bc}	4.35^{cd}
I100/K14//G-4	34.25^{b}	39.75^{de}	2.25	12.75^{bcde}	24.00^{bc}	4.22^{cd}
I100/B54//A-5	36.75^{ab}	48.25^{ab}	3.00	16.00^{a}	30.75^{ab}	5.85^{ab}
I100/B54//A-6	38.75^{a}	46.25^{abc}	2.75	14.25^{abcd}	27.00^{abc}	4.30^{cd}
K/I100//B63///G-7	33.00^{c}	47.25^{abc}	2.25	14.50^{abc}	32.75^{a}	6.55^{a}
K/I100//B63///G-8	34.50^{b}	49.50^{a}	2.25	14.00^{abcde}	30.50^{ab}	6.15^{a}
Argomulyo	33.00^{c}	38.00^{e}	2.25	11.00^{e}	22.75^{c}	4.97^{bc}
Grobogan	33.00^{c}	42.00^{cde}	2.00	13.75^{abcde}	27.25^{abc}	4.30^{cd}

Note: The numbers in the same column followed by the same letter are not significantly different by LSD at 5%

In the character of yield, there were four soybean lines with number of pods significantly higher than Argomulyo and Grobogan varieties (Table 3). The four soybean lines are I100/K8//A-1 (33.00), I100/B54//A-5 (30.75), K/I100//B63///G-7 (32.75) and K/I100//B63///G-8 (30.50). The last three soybean lines were genotypes with grain yield per plant higher when compared to other genotypes. The soybean line K/I100//B63///G-7 produces seed with a weight of 6.55 g per plant, while K/I100//B63///G-8 and I100/B54//A-5 successively produce seeds with a weight of 6.15 and 5.85 per plant.

Discussion

Rust disease symptoms in this study started to appear since 7 to 14 days after inoculation (data not shown). The incubation period in the present study a little longer when compared with the results of other research in Africa. Twizeyimana et al. (2007) found that in Nigeria it took 5 to 7 days after inoculation to lesion of rust disease appear on the surface of leaves. Meanwhile, Maphosa et al. (2013) reported that the incubation period of rust disease in Uganda began to be seen since 4 to 5 days after inoculation. This means that the isolates of rust fungus from Africa are more virulent compared with isolates from Indonesia, and or soybean genotypes from Indonesia are more resistant than soybean genotypes from Africa.

The differences of incubation period that found in this research caused by the differences in environmental factors as well as genetic differences of the plant material used. The optimal environmental conditions required for spore germination and disease perpetuation, i.e.: high relative humidity of at least 85% (Twizeyimana and Hartman 2010) for 14 hours (Nunkumar et al. 2009), and a temperature between 17 and 28°C (Bonde et al. 2007; Del Ponte and Esker 2008). In this study, the lowest relative humidity that recorded during the research was 56%, and the highest was 81%. While the recorded temperature ranges between 22 and 31°C, the environmental condition like this theoretically can not support the spores of *P. pachyrhizi* to germinate and grow to the maximum.

Although the incubation periods of rust disease in present study longer when compared with the results from Africa, the inoculation of rust disease that has been done is able to bring up the different reactions of soybean genotypes tested. The reaction differences seen in the number of lesions between one genotype to other genotype were observed. Lesions of rust disease that appears varies between genotype and within genotype, ranging from 2 lesions cm^{-2} (in line I100/K14//G-3 and K/I100//B63///G-7) to 22 lesions cm^{-2} (line I100/K8//A-1) on the first observation, and from 4 lesions cm^{-2} (in line I100/B54//A-6, K/I100//B63///G-7 and K/I100//B63///G-8) to 25 lesions cm^{-2} (line I100/K8//A-1) on the second assessment (data not shown). Differences in the reaction of genotypes tested are also found in other studies (Pham et al. 2009; Twizeyimana et al. 2008). Pham et al. (2010) stated that genotypes with non-characterized genes for resistance may be useful for host plant resistance studies and breeding soybeans for rust resistance.

The reaction of soybean genotypes with resistance against rust diseases showed that all of the genotypes classified as resistant on the first observation while at the second assessment all of the genotypes categorized as moderately resistant. The different resistance reaction between the first observation and second assessment is caused by spores of the rust disease require time to germinate and formed the new spores. According to Yang (2002), after an infection has occurred, it takes 5 to 7 days to produce uredinia by urediniospores and 10 to 20 days to produce a new generation of spores. This difference gives guidance for soybean breeders to determine the appropriate time to conduct the selection. Sulistyo and Sumartini (2015) found that there are differences in heritability of rust disease severity on observation of one, two and three weeks after inoculation.

The emergence of rust diseases on the various phases of the development of soybean will determine how much yield loss will occur. Kumudini et al. (2008) found that if the rust disease began to occur at the R2 growth stage (full flowering phase), it would cause yield losses up to 66-68%, meanwhile, when it started at the R5 growth stages (seed filling phase), it will cause yield losses reach 35-39%. In this research, a soybean genotype with early flowering can avoid a large yield loss. The mechanism was shown by line K/I100//B63///G-7 and K/I100//B63///G-8. Both of these soybean lines flowering at 33 and 34 DAP (respectively), had the highest seed yield per plant (6.55 and 6.15 g, respectively) compared with other lines. In contrast, the line I100/K8//A-1, I100/K8//A-2 and I100/B54//A-6 were flowering at about 38 DAP, had a weight of seeds per plant (4.70, 3.88 and 4.30 g, respectively) were significantly lower than the two previous line.

Plant height in this study appears to be one of the factors that will determine differences in the severity of rust disease on soybean genotypes tested. Correlation analysis showed that there is a significant negative correlation ($r = -0.753$, $P < 0.05$) and negative correlation ($r = -0.077$, $P > 0.05$) between plant height with the number of rust lesions in the first and second observation, respectively. It means that the higher a plant, then the fewer rust disease lesions. This is not surprising because *P. pachyrhizi* does not have an active mechanism for spreading the spores. According to Isard et al. (2005), wind seems to be critical factors for spread out spores and lifting them out of the canopy. Thus, it takes quite much wind to spread the spores of rust on soybean genotypes with appearances tall plants.

Rust disease in present research did not seem to affect the character of other yield components, such as the number of branches, the number of fertile nodes and the number of pods. However, the three characters have an influence on seed yield per plant. According to Oz et al. (2009) number of pods per plant had significant correlations with seed yield and gave direct positive effect. Valencia-Ramirez and Ligarreto-Moreno (2012) found a similar result with the addition character i.e. the number of nodes per plant. Malik et al. (2007) suggested that number of pods can be considered as selection criteria in improving the bean yield of soybean genotypes.

The evaluation of resistance of soybean genotypes against rust disease in this study showed that no soybean genotype classified as immune or resistant genotype to rust disease. The whole genotypes tested were categorized as moderately resistant genotype. Days to flowering and plant height influence the development of rust disease severity. Three of the eight lines produce seeds with the seed weight per plant heavier than Argomulyo and Grobogan varieties, namely K/I100//B63///G-7, K/I100//B63///G-8 and I100/B54//A-5. Characteristics of the three genotypes, among other the performance of plants is high with a lot of number of fertile nodes and number of pods.

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