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Characterization of soybean genotypes for Asian soybean rust reaction under screen house condition

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Abstract. *Inayati A, Yusnawan E. 2016. Characterization of soybean genotypes for Asian soybean rust reaction under screen house condition. Biodiversitas 17: 609-613.* Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* is one of the major diseases limiting soybean yield. This disease has widely spread on soybean crops in Indonesia. The use of resistant cultivars is one of the economical approaches to control ASR. The objectives of this study were to assess the resistance of soybean lines derived from crossing two large-seeded cultivars (Baluran and Grobogan) with a broad adaptive cultivar (Kaba) and to identify resistant genotype characteristics under screen house conditions. All genotypes were artificially inoculated with *P. pachyrhizi* uredospores. Number of pustules per leaf, the development of ASR, and yield components including number of intact pods per plant, number of empty pods, and weight of pods per plant were observed. Thirteen lines of Baluran pedigrees had higher resistant response to ASR compared to Grobogan pedigrees. Fewer numbers of pustules (8 pustules cm^{-2}), lower value of area under the disease progress curve (AUDPC), and redish brown (RB) lesion type were observed in resistant lines. In contrast, susceptible lines had more pustules (> 21 pustules cm^{-2}), higher AUDPC value, and had mixed lesion type (RB and Tan). ASR reduced seed size and yield. The average weight of 100 seeds of resistant lines was 10.2 g while on susceptible lines, the 100-seed weight ranged from 8.7 to 12.6 g. The average yield per plant varied from 2.7 to 6.1 g. Baluran/Grobogan pedigrees were more susceptible to ASR than Baluran/Kaba pedigrees, however, those pedigrees showed better yield per plant and were supposedly more tolerant to ASR.

Keywords: Asian soybean rust, genotype, resistant, soybean, susceptible

INTRODUCTION

Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* is one of the important pathogens which reduces soybean yield (Semangun 1993). This pathogen has widely spread on soybean crops from subtropics to tropical regions. In Indonesia, ASR infection had been reported since 1991 (Semangun 1993). This disease emerges in every season, especially at the end of rainy season (T > 28°C, RH > 95%) (Sumartini 2010). ASR is considered the most destructive soybean foliar disease (Li and Young 2009, Miles et al. 2003). Heavily infected crops result premature defoliation which effect on pod filling (Kumudini et al. 2008; Ribeiro et al. 2009) and reduce number of pods as well as seed size (Diaz et al. 2007). Significant yield loss occurs on early infected soybean crops than late infection. Severe infection significantly reduces the yield up to 80% (Twizeyimana et al. 2008).

The main symptom of infected soybean crops is the lesions on leaves, which consist of pustules containing large numbers of uredospores. In sub tropical regions, the first symptom appears after flowering (R1 to R3) on the leaves in the lower canopy (Faske et al. 2014). However, in tropical areas such as in Indonesia, rust disease appears at three or four weeks after planting (V3 to V4) (Sumartini 2010). Fungicide application, the use of resistant cultivars, and cultural practice are basic management for reducing soybean rust epidemics (Rupe and Sconyers 2008). Fungicide applications are an effective control for short period, however, not effective for long term management. Several fungicides effective to control soybean rust are from groups of chloronitriles, strobilurins, and triazoles (Muller 2007; Rupe and Sconyers 2008). The use of resistant cultivars and good cultural practices are considered more promising.

Recently, soybean resistant cultivars to all isolates of *P. pachyrhizi* have not been available yet (Bonde et al. 2006, Goellner et al. 2010). Factors affecting the susceptibility of resistant cultivars are durability of the resistance to ASR which is easily broken due to pathogen variability (Oliveira et al. 2005), the complexity of genes that controls resistance to ASR, and environmental factors (Garcia et al. 2008). Thus, tolerant cultivars are more reasonable for ASR integrated control (Twizeyimana et al. 2008). Tolerance can be defined as plant capacity to resist pathogen infection and development, without significant reduction in yield or quality of the product (Schafer 1970). Three different reaction types may occur on soybean in response to *P. pachyrhizi* infection based on lesions types, i.e. (i) immune reaction (IM) without visible lesions, (ii) a resistant reaction with reddish-brown (RB) lesions, and (iii) a susceptible reaction with tan (TAN) lesions (Bromfield 1984; Goellner et al. 2010).

The best possible techniques to develop new cultivars resistant to rust diseases are to screen soybean germplasm and to create pedigree derived from the existing cultivars and lines. Studies to estimate yield losses caused by this disease are also important, so future cultivation of such cultivars suffering from huge yield losses could be avoided. Cultivars with larger seed size, early maturity, high yield and having broad adaptation have become a main goal of Indonesian breeder program to reduce soybean production gap and the national demand. Until 2011, Grobogan is the only superior cultivar in Indonesia which has those ideal characteristics: large-seeded, early maturity and high yield. However, Grobogan has limitations due to less adaptation to large area and only specific to certain locations. Crossing between Grobogan with broad adaptive cultivars, namely Kaba and Malabar is promising to develop new cultivars which have large-seeded, early maturity, high yield and broad adaptation. This study therefore, aimed to assess the resistance of soybean breeding lines derived from crossing of two large-seeded cultivars (Baluran and Grobogan) with a broad adaptive cultivar (Kaba), and to identify the characteristics of resistant lines under screen house conditions.

MATERIALS AND METHODS

Plant materials and experimental design

Thirteen soybean genotypes derived from crossing Baluran, Grobogan, Kaba, and Malabar cultivars were planted in Indonesian Legumes and Tuber Crops Research Institute (ILETRI) screen house at Kendalpayak, Malang, East Java, Indonesia. The experiment was arranged in completely randomized design (CRD) with triplicate. Each pot consisted of two plants and every soybean genotype was planted on six pots.

Pathogen isolate and inoculation procedure

Uredospores of *P. pachyrhizi* were used as inoculum. The uredospores were harvested from susceptible cultivar (Ringgit) which was cultivated in a screen house as a source of inoculum. Ringgit cultivar was planted a month prior to the study. The average temperature during study was maintained at 25-28°C, and the relative humidity was 80-85%. Prior to inoculation, infected leaves of Ringgit were harvested and placed on plastic trays and incubated for 24 h. Uredospores were removed from the leaves with a paintbrush. Inoculum was prepared by suspending uredospores in water and 20 µL Tween 20. The suspension was mixed well, and filtered through cheese cloth. Uredospore concentration was adjusted to 10^4 spores mL^{-1}. The uredospores were sprayed on healthy soybean breeding line leaf surface in the evening at 4 pm with a hand sprayer. Inoculation was conducted twice at three and four weeks after planting (WAP) (Sumartini 2010; Inayati and Yusnawan 2016).

Table 1. The resistance level of soybean lines to ASR (Shanmugasundaram 1977)

Resistance criteria	**IWGSR Score**
Immune (I)	111
Resistant (R)	122, 123, 132, 133, 222, 223
Moderately resistant (MR)	142, 143, 232, 233, 242, 243, 322, 323
Moderately susceptible (MS)	332, 333
Susceptible (S)	343

Disease rating and data analysis

Response of soybean lines to ASR was evaluated starting from seven days after inoculation (DAI). ASR infection level and the resistance of soybean lines were rated using modified three digits of IWGSR (International Working Group on Soybean Rust) (Shanmugasundaram 1977) (Table 1). The first digit denotes the upper position of the most diseased leaves in the leaf canopy of the plant, where 1 = bottom third of the leaf canopy, 2 = middle third of the leaf canopy, and 3 = upper third of the leaf canopy). The second digit denotes the density of rust lesion on the most diseased leaves, where 1 = no pustules, 2 = light pustules density (1-8 pustules cm^{-2}), 3 = medium pustules density (9-16 pustules cm^{-2}) and 4 = heavy pustules density (> 16 pustules cm^{-2}). The third digit denotes the infection type on the most diseased leaves, where 1 = no pustule, 2 = no sporulating pustules, 3 = sporulating pustules. Disease progress was quantified by calculating the Area Under Disease Progress Curve (AUDPC) according to Simco and Piepho (2012). Yield components consisting of filling pods, empty pods, and weight of 100 seeds were observed to evaluate the effect of ASR to the yield. Analysis of variance followed by least significant different test (LSD, $p < 0.05$) was performed to determine the difference among genotypes.

RESULTS AND DISCUSSION

Results

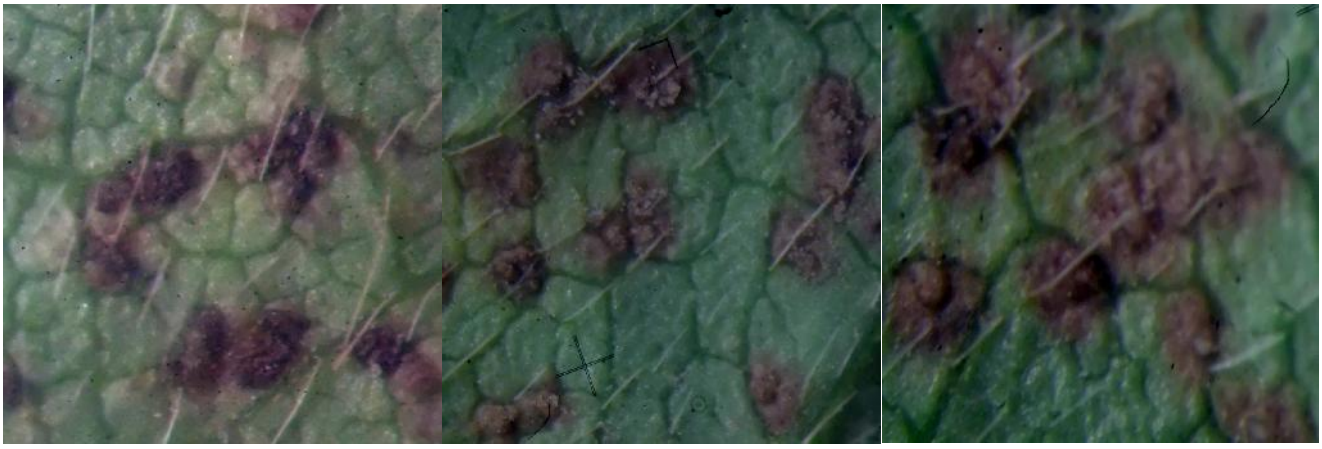
Soybean genotype response to ASR varied in relations to the number of pustules, lesion types, and the disease progress (AUDPC). The number of pustule increased in line with the plant age and the duration of infection (Table 2). Significant difference ($p < 0.01$) in the number of pustules at 42 and 49 DAI and AUDPC of lesion density of soybean lines was observed. In general, majority of Baluran/Grobogan pedigrees showed higher number of pustules than those of Baluran/Kaba. From the data collected at 21 DAI, 11 lines had light pustule density (1-8 pustules cm^{-2}) and 2 lines had medium pustule density (9-16 pustules cm^{-2}). The average number of pustules at 49 DAI ranged from 8 to 24 pustules cm^{-2} and *P. pachyrhizi* had covered almost the whole plants. Nine of thirteen lines had the average number of pustules more than 16 pustules cm^{-2} and were categorized as susceptible (S). The positive correlation between AUDPC and pustule number ($r = 0.73$, equation not shown) was observed related to rapid advance to the disease progress. This caused by the increased number of pustule as a source of inoculums which resulted more secondary infections. Three different lesion types as a response to ASR were observed in this study (Figure 1). Most of the genotypes showed RB lesion type (Table 2), and only one genotype showed T and mixed (RB/T).

Phakopsora pachyrhizi infection reduced yield, yield components, and seed size (Table 3). Even though there was no significant difference on the number of intact pods between the susceptible and resistance genotypes, the average number of intact pods on susceptible genotypes was fewer than the resistant breeding lines. The intact pod

Table 2. Number of pustule cm^{-2}, score, resistant criteria, lesion type, and AUDPC values on large-seeded soybean lines

Large-seeded soybean lines	Number of pustules per cm^{2}			IWGSR score	Resistant criteria	Lesion type	AUDPC
	21 DAI	42 DAI	49 DAI				
Bl/Kb1	9.00^{a}	14.00^{a}	14.83^{cd}	333	MS	RB	131.88^{ab}
Bl/Kb2	10.17^{a}	12.67^{ab}	14.17^{cd}	333	MS	RB	112.04^{ab}
Bl/Kb3	7.33^{a}	11. 67^{ab}	8.17^{d}	223	R	RB	55.21^{b}
Bl/Gr15	7.50^{a}	9.83^{ab}	20.83^{abc}	343	S	RB	159.50^{ab}
Gr/Bl16	8.33^{a}	12.83^{ab}	24.67^{a}	343	S	RB	227.00^{a}
Bl/Gr19	8.67^{a}	11.00^{ab}	19.33^{abc}	343	S	T	103.33^{ab}
Bl/Gr21	5.67^{a}	10.50^{ab}	21.00^{abc}	343	S	RB	150.54^{ab}
Bl/Gr23	8.50^{a}	8.50^{b}	20.17^{abc}	343	S	RB	91.04^{ab}
Bl/Gr51	5.17^{a}	10.33^{ab}	18.00^{abc}	343	S	RB	88.59^{ab}
Bl/Gr58	5.17^{a}	14.00^{a}	16.00^{bcd}	333	MS	RB	177.50^{ab}
Bl/Kb66	6.00^{a}	10.17^{ab}	23.33^{ab}	343	S	RB/T	202.67^{ab}
Bl/Gr70	6.67^{a}	13.00^{ab}	21.50^{abc}	343	S	RB	179.34^{ab}
Bl/Kb71	6.83^{a}	11.00^{ab}	21.17^{abc}	343	S	RB	187.13^{ab}

Note: R: resistant, MS: moderately susceptible, S: susceptible, RB: reddish brown, T: tan, RB/T: mixed reddish brown and tan. Number at the same column followed by the same notation was not significantly different based on LSD test ($p < 0.05$).



A B C

Figure 1. Lesion type of ASR on large-seeded soybean lines. A. Reddish Brown, B. Tan, and C. Mixture of Reddish Brown and Tan

Table 3. Number of intact pods, number of empty pods, weight of 100 seeds and yield of large-seeded soybean lines infected by *P. pachyrhizi*.

Large-seeded soybean lines	Number of intact pods	Number of empty pods	Weight of 100 seeds (g)	Yield per plant (g)
Bl/Kb1	18.58^{a}	5.50^{a}	9.97^{bc}	3.07^{b}
Bl/Kb2	19.42^{a}	5.25^{a}	8.74^{c}	2.79^{b}
Bl/Kb3	22.83^{a}	6.83^{a}	10.22^{bc}	4.37^{ab}
Bl/Gr15	19.42^{a}	5.42^{a}	11.61^{ab}	4.43^{ab}
Gr/Bl16	24.00^{a}	3.58^{a}	11.16^{ab}	5.10^{ab}
Bl/Gr19	25.08^{a}	5.00^{a}	11.67^{a}	6.11^{a}
Bl/Gr21	22.00^{a}	4.92^{a}	11.66^{c}	5.22^{ab}
Bl/Gr23	17.33^{a}	2.00^{a}	10.18^{bc}	3.49^{ab}
Bl/Gr51	21.67^{a}	6.33^{a}	9.77^{c}	4.15^{ab}
Bl/Gr58	18.92^{a}	4.92^{a}	12.52^{a}	4.63^{ab}
Bl/Kb66	25.67^{a}	5.83^{a}	10.25^{bc}	4.29^{ab}
Bl/Gr70	19.50^{a}	4.25^{a}	12.64^{a}	4.89^{ab}
Bl/Kb71	23.75^{a}	3.50^{a}	10.52^{ab}	4.92^{ab}

Note: Number at the same column followed by the same notation was not significantly different based on LSD test ($p < 0.05$)

numbers varied from 17 to 25 pods. In contrast, the number of empty pods on resistant genotypes was the highest. However, there was no significant difference on the number of empty pods between the susceptible and resistant genotypes. The average yield per plant was low due to the ASR infection. The yield varied from 2 to 6 g and the average weight of 100 seeds on resistant lines was 10.2 g while on susceptible lines, that variable ranged from 8 to 12 g (Table 3).

Discussion

The genotype characteristics resistant to ASR in the present study had lower number of pustules, lower AUDPC value, and reddish brown (RB) lesion type, in contrast to susceptible genotypes which presented severe symptoms and more rapid disease progress. The intensity of uredospore sporulation and periodical severity assessment were important parameters for classification of genotypes into tolerant or susceptible to ASR (de Araujo and Vello 2010). Large variation was observed in the AUDPC, even though lack association was noticed between AUDPC value and the resistance of large-seeded soybean genotypes to ASR. The AUDPC value was influenced by genotype x environment interactions as explained by Steffenson and Webster (1992) and Cherif et al. (2010). They reported that relationship between the final disease severity and the AUDPC was highly influenced by the environment. In addition, they noted that high values of apparent infection rate could occur sometimes on genotypes with reduced disease severities when there was rapid increase of pathogen infection from a low to a moderate level within a short period. A positive correlation between number of pustule and AUPDC value suggested that genotypes which had plenty of pustules possessed faster disease progress. This could imply that the resistance mechanism present in these genotypes respond rapidly once the rust pathogen established in the host cells as observed in the present study.

RB lesions are formed because of the hypersensitive response of the soybean crops to *P. pachyrhizi*. This reaction will inhibit the fungus development. Lesion color is known to be controlled by resistance genes of Rpp2 and Rpp4, thus it should be considered when selecting resistant genotypes (Yamanaka et al. 2010; 2013). On susceptible lines, lesion was clearer (Tan) and some were mixture between RB and Tan, even though most of the lesion type in this study was RB. However, the development of the pathogen which represented by the number of pustules was still high, resulting variation of lesion color. Variation among genotypes makes the difficulty for grouping all phenotypes into a limited number of lesion types, such as RB (Resistant) and TAN (Susceptible) (Kato and Yorinori 2008).

ASR reduced yield, yield components, and seed size since *P. pachyrhizi* infection initiated early defoliation which effected on pod filling (Kumudini et al. 2008; Ribeiro et al. 2009) and reduced number of pods and seed size (Dias et al. 2007). Foliar pathogens were not only impairing the healthy green leaf area of crops, but also influencing the photosynthetic activity of the healthy (green) parts of the leaves (Kumudini et al. 2008). The seed size represented by the average weight of 100 seeds was categorized as medium seeds. In this study, the average weight of 100 seeds on resistant lines was 10.2 g while on susceptible lines, that parameter ranged from 8.7 to 12.6 g.

A study conducted by Ahmad et al. (2010) on leaf rust infected wheat showed that cultivars or genotypes in which AUDPC was maximum, the yield losses were also maximum. Cultivars which performed lower AUDPC value, these cultivars suffered from less yield losses (Ahmad et al. 2010). Dissimilar response was observed on ASR, the increase value of AUDPC was not linearly followed by the maximum reduction of the yield. In the present study, AUDPC value is possibly highly influenced by genotype factor. As explain by Pham et al. (2010), the response of the genotypes which were controlled by Rpp genes to soybean rust was dependent on the experiment and the time of the trial were conducted.

In conclusion, response of soybean breeding lines to ASR showed that Baluran/Grobogan pedigrees were more susceptible to ASR than Baluran/Kaba pedigrees. On the other hand, Baluran/Grobogan pedigrees showed better yield per plant, and were categorized as tolerant lines.

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