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Disease severity and infection rate of soybean leaf rust disease on several genotypes

Mohammad Setyo Poerwoko[1], Endang Budi Tri Susiliwati [2], Moh. Rizal Pahlevi [2]

[1]Agronomy Study Program, Plant Breeding Spcialist, Agriculture Faculty, Jember University Kalimantan Street 37, Tegal Boto Campuss, Jember 68121 East of Java, **Indonesia.**
[2]Protected Study Programme, Agriculture Faculty, Jember UniversityKalimantan Street 37, Tegal Boto Campuss, Jember 68121 East of Java, **Indonesia.**

***Correspondence:** moh_setyo_poerwoko@yahoo.com **Accepted:** 18 Nov. 2017 **Published online:** 19 March 2018

Soybean leaf rust disease caused due to *Phakopsora pachyrhizi* Syd so far is considered as a major disease of soybean production. The utilization of soybean genotype with high yielding and source of genetic resistances to soybean leaf rust is could be one one effort to overcome the low production problem. Resistant varieties requires a source of resistant parent. Evaluation of these variety of the disease and the rate of infection is important as an indicator to assess the viability of a genotype, as a source of resistant gen. Based on IWGSR (International Working Group Soybean Rust) rating codes, from 12 genotypes tested (four parents and 8 crosed genotypes), only one genotype resulted from crossing between Mutiara-1 and GHJ-6 was categorized resistant. These genotypes had the lightest lower of disease index (KP) with the lowest infection rate (r), become the most elegible source of gamatic resistace. The remaining 11 genotypes were moderatly resistant to disease. Genotypes GHJ-6 (parent), Mutiara-1 (parent), crossing of GHJ-7 and Mutiara-1, and the crossing of Mutiara-1 and GHJ-6 had same rate of infection (54.46) with the crossing of Mutiara-1and GHJ-6 (r=0.22) but showed lower and considered as source resistant genes. Another indicator that can be used to evaluate this eligibility of genotype as a source of resistant genes is a decrease in crop yields due to soybean leaf rust infections.

Keywords: Soybean leaf rust disease; genotype; severity of disease; infection rate

INTRODUCTION

Soybean is the second important food crop in Indonesia because of its high nutriti-onal value, particularly as a source of protein (Suprapto, 2004). Soybean seeds contain protein (40%) and vegetable oil (20%). It is the raw materials for many traditional foods, e.g. tempeh (fermented soybean cake), tofu, soy sauce, butter, and oil, as well as in the manufacturing of medicinal and industrial products (Adisarwanto, 2006). However, some factors like, lack of economy, low price and biotic yield reducing (disease and pest) factorss become the major contrains of soybean production and productivity.

Soybean leaf rust caused due to *Phakopsora pachyrhizi*, Syd is one major disease of limiting soybean production. The decrease is due to disturbance in the process of photosynthesis. a severe disease attack results in the failure of pods filling (Suprapto, 2004). A common symptom includuding viability of uredospora pathogen particularly on the bottom side of leaves (BPTP Bogor-JICA, 1990). Among several means, identifying the sources of gametic resistant to overcome the leaf rust disease on soybean could be the sustainable and moswt efective approach.
Soybean genotypes, namely Strain Jember (GHJ) which consists of GHJ-2, GHJ-3, GHJ-6, GHJ-7,

and GHJ-10, are moderately resistant to leaf rust (Amarullah, Trisusilowati, & Purwoko, 2009). Genotypes, GHJ-6 and GHJ-7 were used as materials for varietal research. In the subsequent breeding program. Leaf rust resistant varieties of *Mutiara-1* and *Slamet* were used as donor parent to improve their resistance toward development of resistant high yielding varieties. The characteristic of the breeding materials were shown in Table 1.

The severity and rate of infection of leaf rust disease of soybean in tested genotypes showed inherited resistant properties of parent. efforts to overcome the disease can be done with the use of novel resistant varieties, with high production for leaf rust severity of soybean. Two new soybean genotypes GHJ-6 and GHJ-7 are reported to have high production and resistance to leaf rust moderately resistant criteria. ased on disease severity and infection rate, degree of resistace against leaf rust of soybean were evaluated for genotypes obtained from crossing between GHJ-6 and GHJ-7 with the donor parent Mutiara-1 and Slamet. The evaluation results can be used as an indicator of the feasibility of genotype with the same degree of resilience to be elected as a source of resistance genes, and to determine the degree of resilience from the soybean genotypes tested.

The results of the study can be used as reference material especially for the soybean plant breeders in an effort to get hybrid soybean varieties that are resistant to leaf rust disease. The disease symptoms appear on the leaves, stems, and sometimes on the trunk.

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First appeared on the leaf surface in the form of small patches of grey or brown or dark brown (Semangun, 1990). Patches of rust are visible before pustules broke. Spotting looked angled corners, because it is restricted by the leaf vein near the infection site. During flowering period, splotches become larger or sometimes unite and become dark brown and even black (Figure 1b). In general, symptoms of rust initially appear on the undersides of leaves, which then evolved into the leaves upper part later contain uredium formed under the epidermis, when viewed from the top round or oblong. In the center of the uredium holelike structure formed to relese that urediospora.

Urediospora pathogen which formed in uredium are oval-shaped with jagged surface and golden yellow to light brown. Uredium diameter ranges from 5-14 µm to 25-50 µm, 15-25 µm and a diameter urediospora to 18-34 µm (Figure 1). Urediospora finally developed in to teliospore formation is rarely found in the field and does not act ads the initial inoculum for the spreadof the disease (Sumartini, 2016)**.**

Soybean leaf rust pathogens are obligate parasites, and cannot survive in the seeds, but it can survive in the leaf tissue for 40-60 days and 10-40 days in the soils. *P. pachyrhizi* was reported to infect legume crops in the field, namely beans *asu* (*Calopogonium mucunoides*), bean sword (*Cana-valia gladiota*), kratok (*Phaseolus lunatus*), green beans (*Phaseolus vulgaris*), winged bean (*Psophcarpus tetragonolobus)*, green beans (*Vigna radiata L*.), and beans (*Vigna unguiculata*). In addition there are two other types of plants, namely yam (*Pachyrhizus bulbosus*) and pea (*Pisum sativum*) (Semangun, 1990).

Response Genotype Resistance to Soybean Rust Soybean Leaves

The host resistance trait against pathogen is properties owned by plant and is an inherited trait also affecting the level of crop damage caused by pathogens attack (Painter, 1951). The resistance trait of a plant is a multiplicity factors, or a group of factors in the plant which naturally inherited, and are the trait to determine resisting, prevent, or tolerate pests and disease pathogen (Sidiq, 2009).

Table 1. Characteristic of soybean genotype as of the parent varieties crossing

Soybean Genotype	Genotype Characteristic
GHJ-6	Originally called UNEJ-2, has potential to produce result high and resistant to leaf rust.
GHJ-7	NSP originally called, has potential production result high. Has moderately resistant to leaf rust disease.
Mutiara-1	Resistant to rust and leaf spot brown leaf blight
Slamet	Resistant to leaf rust disease

The resistance of varieties (genotypes) plant against a pathogen infection is vary, ranging from very susceptible highly resistant (Semangun, 1990). The degree of resistance of a variety to leaf rust disease of soybean can be assessed based on the criteria according to IWGSR (International Working Group Soybean Rust) (Hardaningsih, 2011).

In general mechanisms of plant resistance to pathogens can be divided into three, namely mechanical resistance, chemical, and functional. Plants have a mechanical resistance in the form of morphologic structure which makes it difficult infected with a pathogen, e.g. thick epidermal cuticle, and the present of wax layer. Resilience chemical resistance is obtained through acids, esters, phenols, and tanner in plant tissue hibited pathogen development. Resilience functional is a temporary endurance that usually occur in plants which essentially vulnerable, but the plant has properties that allow it to heal parts of infected plants, able to avoid the attack of pathogens, or it can pass through a phase vulnerability when no pathogens or when the environment is not suitable for the development of pathogens (Semangun, 1996).

Based on the composition and the number of genes, 3 kind of (1) monogenie, resistant properties are controlled by a single gene dominant or recessive, (2) oligogenik, resistant trait is controlled by several genes which reinforce each other, and (3) polygenic, resistant trait controlled by many genes that each add and each gene react differently to the races or strains of pathogens that result in the emergence of a broad resistance. Genetic resistance is also divided into two types (1) resistance of the vertical, resistance against just one biotype disease, and usually are very resistant, but easily broken by the emergence of new biotypes, (2) resistance of horizontal or general resistance, resistance to many biotypes of diseases with a degree of resilience moderately resistant (Triharso, 1996)**.**

Figure 1a. Symptoms of soybean leaf rust

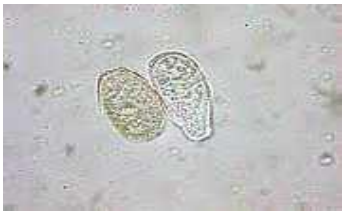

Figure 1b. Urediospora *P.pachyrhizi*

Figure 1 The symptoms of soybean leaf rust and Urediospora *P. pachyrhizi* (Plant Management Network, 2005)

MATERIALS AND METHODS

Research conducted at the experiment station of Polytechnic of Jember, for three months in May to July 2014. Preparation in these experiments include tillage in the experimental area which. The plots size was 2 x 4 m^2 which containing 10 plants.

Seeds used in this study are twelve soybean genotypes consists of four parent i.e. GHJ-6 (G1), GHJ-7 (G2); Mutiara-1 (G3) and Slamet (G4) as the donor parents and eight genotypes result of crossing between four parent, i.e. G1 x G3 (G5), G1 x G4 (G6), G2 x G3 (G7), G2 x G4 (G8), G3 x G1 (G9), G3 x G2 (G10), G4 x G1 (G11), and G4 x G2 (G12). Experiments were conducted in multiple stages. The experiment is based on a randomized block design (RBD) consisted of 12 genotypes each with three replications.

The experiment was conducted in the dry season after paddy harvest. Response or

resistance properties of eight soybean genotypes from crossing between four previous

Table 2. Degrees of soybean genotypes resistance against leaf rust soybean based code Rating IWGSR (Yang, 1977)

Scale	The degree of Resistance	Rating code IWGSR
1	Imun	111
2	Resistance	122, 123, 132, 133, 222, 223
3	Moderatly Resistance	142, 143, 232, 233, 242, 243, 322, 323
4	Moderatly Susceptible	332, 333
5	Susceptible	343

Test of significant difference between the treatment were analyzed by Duncan's Multiple Range Test (DMRT) at the level of 0.05

generation (G1, G2, G3, and G4) to leaf rust disease of soybean, *P. pachyrizi*, tested in the field through the transmission of the disease naturally. During the research process, crop management were conducted; i.e. planting, fertilizing, thinning, weeding, and the controlling of the factors that can cause damage to the plant, as commonly work out in the soybeans cultivation.

The rate of infection. The development rate of infection which supports the spread of the disease was measured using the epidemiology formula (Van Der Plank, 1963)**,** namely:

$$Xt = Xo\ e^{rt}$$

Where,

Xo = proportion of the disease at the beginning of the epidemic

Xt = proportion of the disease after a course of epidemics in time t e = 2.71821 (Constants)

R = the rate of infection

T = the time course of the epidemic. One week after the earliest symptoms appear, the value KP soybean leaf rust at all genotypes tested showed significant difference among the treatments

Variable observations used in these experiments include:

Soybean Rust Symptoms

Observations to detect the occurrence of soybean leaf rust infection on each genotype, carried out every day until the emergence of the earliest symptoms of the disease as a basis to determine the incubation period of the diseases severity of infection.

Evaluation of the severity of infection in each genotype conducted one week after the appearance the first symptoms, and the second observations with an interval of one week. By determining the severity of disease (KP) and the rate of infection (r). Severity of disease (KP) is determined by using the formula Townsend and Heuberger (Sinaga, 2003) which describes the density patches of rust on the leaves of 1 cm^2 samples, and (3) the third digit on patches of rust formed pustules and occurred sporulation which resulted urediospora. The degree of resistance genotypes then catagorized using a scale of 1-5 IWGSR into five groups as shown in Table 1.

The Degree of Resilience.

Code rating IWGSR to determine the degree of resistance genotype is determined by a combination of three digits, namely (1) The first digit indicates the location of the leaves sample at the plant measured from the ground, (2) the second digit describe density patches of rust on 1 cm^2 leaves samples, and (3) the third digit on pustule formed postul and sporulation occur that produce urediospora. The degree of resistance then categorized using a scale of 1-5 IWGSR into five groups as shown in Table 2.

RESULTS

Twelve soybean tested genotypes are four genotypes parent and eight genotypes offspring from crossing between parent. The field trial was conducted 79 days after planting (DAP) by inoculation of pathogenic leaf rust naturally. All plant can be infected and show symptoms of leaf rust disease, and significantly different between treatments.

The earliest symptoms of disease appeared in the age of 65 DAP, and occurs only in G2 and G3 genotypes (Table 3). In other geno-types the symptoms vary ranging between 65-68 days after planting, and most symptoms come late in G1 and G10.

Table 3. Symptoms of leaf rust appears on the earliest soybean plant, severity of disease, and the rate of infection

Genotype	Most symptoms SRL	Increase severity (%) and the rate of infection (unit/ day)		
	Early appear on Plant age (dap)	KP in plants age 72 dap (%)	KP in plants age 79 dap (%)	The rate of infection (unit/day)
G1 (GHJ-6)	68	39.92 c	45.86	0.02
G2 (GHJ-7)	65	53.23 a	63.73	0.03
G3 (Mutiara-1)	65	48.80 abc	55.83	0.02
G4 (Slamet)	66	51.18 ab	62.28	0.03
G5 (1x3)	66	45.19 abc	51.97	0.02
G6 (1 x4)	67	46.87 abc	54.46	0.02
G7 (2 x3)	66	41.20 b-e	47.96	0.02
G8 (2 x 4)	66	49.56 abc	61.67	0.03
G9 (3 x1)	67	41.66 b-e	47.48	0.02
G10 (3 x2)	68	38.92 c	43.89	0.02
G11 (4x1)	67	44.87 abd	51.07	0.02
G12 (4x2)	66	53.65 a	66.48	0.03

SRL = Soybean rust leave; KP = severity of disease; Figure in the same coloumn followed by the same letter show no significant difference in the extent of 0.05 DMRT test.; dap = day after planting

KP = [S (n x V) / Z x N] x 100%
KP = severity of the disease, where,
n = number of infected leaves on each scale,
v = value of disease severity scale,
V = the value of the highest scale,
N = number of leaves were observed.

Table 3 revealed that the value of KP was found lower (38.92%) in crossbred of Mutiara-1 with GHJ-7 (G10) followed by crossbred Slamet with GHJ-7 (G12). With KP value of 53.65%. In the four genotypes parent used as a comparison, the value of KP lightest 39.92 %. The percent GHJ-6 (G1) had the lower KP value 39.92% and GHJ-7 (G-2)and the most severe GHJ 53.23 percent on GHJ-7 (G2) had higher disease severity of 53,23%. The lower the KP value, the higher the resistace response from plant was observed. The test results showed that G10 with the lower KP value not significantly different compare to Mutiara-1 (G3) as a parent but significantly different with GHJ-7 (G2). By comparison, G10 was also not significantly different to G1.

Judging from the value of KP and the response of resistance genotypes to leaf rust from the cross between tested parents, the G1 and G3 can be used as one parent in crosses which gives better results against leaf rust severity. The disease severity was found in increasing order in nlater deays. A large increase in the value of KP in the tested genotypes up to age 12 week after planting plants ranged from 4.9 up to 12.1 %. KP value on the age of 79 DAP did not change or persist in the same genotype as the plant ages of 72 DAP, respectively in G10 (43.89 %) and G12 (66, 48 %).

The tested Genotype G10 had 0.02 units/day infection rate with mild KP value. Genotypes with the highest infection rate occurred in the three genotypes, namely G2, G8 and G12 each with an infection rate of r = 0.03 units/day. The small value of r on genotype G10 shows that these genotypes have a resistance response that is more resistant to leaf rust pathogen infection compared with other genotypes.

Table 4. The degree of resistance of soybean genotypes to leaf rust disease of soybean Based code rating IWGSR

Genotype	Soybean genotypes degree of resistance in plant age 72 dap	
	Rating code IWGSR[1)]	Degree of resistance
G1 (GHJ-6)	233	MR
G2 (GHJ-7)	243	MR
G3 (Mutiara-1)	233	MR
G4 (Slamet)	233	MR
G5 (G1 xG3)	233	MR
G6 (G1 x G4)	233	MR
G7 (G2 x G3)	233	MR
G8 (G2 x G4)	233	MR
G9 (G3 x G1)	143	MR
G10 (G3 x G2)	133	R
G11 (G4 x G1)	233	MR
G12 (G4 x G2)	233	MR

IWGSR =International Working Group soybean rust; dap = day after planting ; MR = Moderatly Resistance; R = Resistace

Based on the IWGSR code rating, with the exception of G10 (relatively resistant), all genotypes categorized as having degree of resilience somewhat resistant. Genotype G10 in Table 3 were categorized as having degree of resilience resistant, based on the code rating IWGSR with a combination of 133 means that the first digit numeral 1 indicates the leaves of the plant height one-third of the land surface of infected leaf rust, the second digit indicates the number 3 spot of rust on the leaves infected with medium density per cm^2 leaf (9-16 spots/cm^2), and the third digit, figure 3 shows that on patches of rust pustules have occurred sporulation.

Based on the value KP and the rate of infection, the 11 tested genotypes show the degree of resilience and categorized as moderately resistant. Four parent genotypes, that are G1 (GHJ-6), G2 (GHJ-7), G3 (Mutiara-1), and G4 (Slamet) are eligible to be selected as source of resistant genes, i.e. G1 (KP = 39.92 %, the rate of infection of 0.02 units/day) and G3 (KP = 48.80 %, the rate of infection of 0.02 units/day). Seven other genotypes (the result of crossing between four parent) with a degree of resilience is categorized as somewhat resistant. The test results showed that the G10 with lightest KP value did not differ significantly with G3 as a parent but significantly different from G2. The G10 genotype was also not significantly different to G1.

One week after the appearance of first symptoms, the plant aged 72 DAP an increase KP occurred in all tested genotypes (Figure 2). Increase in KP value occurs because of the availability of sources of inoculums that growing on the infected plants in the field. Leaf rust pathogen generally prefer to attacks the older leaves. (Soesanto, Utami, & Tarjoko, 1994) also argued that the older soybean crop will be more susceptible to leaf rust.

Seven other genotypes (offspring from 19 cross pollination of four parents) categorized as moderately resistant, and eligible to be selected as source of resistant gene. Genotype G7 (the result of crossing GHJ-7 with Mutiara-1) and G9 (the result of crossing the Mutiara-1 with GHJ-6) with KP value of 41.20 percent and 41.66 % with the same value of r = 0.02 units/day were showed resistant response. The trial also showed a change in degree of resistance of soybean varieties to leaf rust pathogen, that are G3 and G4. Based on the year of 1918-2005 soybean description the were resistant to leaf rust (Suhartina, 2005). On this trial they show in the categories of moderately resistant. Birawati (Hardaningsih & Pusposendjojo, 1986)

Changes in the degree of resilience can be affected by several factors, especially the differences in environmental conditions and leaf rust pathogen virulence in the test location. Environmental factors that influence the development of the disease are mainly the change on the degree of genotype resilience, as factor of temperature and moisture. At a certain range, the temperature can decrease the level of resistance, such as the horizontal and vertical resistance formed by major genes. If the temperature conditions favor the development of pathogens then pathogen can complete cycle of the disease in a short time while still providing opportunities for epidemic diseases. Excessive moisture and prolonged or occur repeatedly in the form of rain or dew is a factor that greatly helped the development of epidemic diseases, and can cause a host plant into succulent and susceptible to support increased sporulation of fungal pathogens to produce spores to the surface of the host body and provide opportunities spores germinate so the impact of the changes in the degree.

In addition to these two factors, the growing media should be taken into consideration. The environmental factors may influence the development of disease and the impact of disease severity.

Based on IWGSR rating codes, from 12 tested genotypes (four genotypes parents and eight genotypes resulted from crossing between these), only one genotype resulted from crossing between Mutiara-1 and GHJ-6 was categorized resistant. In addition to resistance, this genotype is the lightest severity of disease (KP) and the lowest infection rate (r), thus it can be said that it is the most eligible source of resistant genes to be selected for the breeding of resistant varieties. Other 11 genotypes belonged to intermediate resistant and between those 11 genotypes, four genotypes i.e. GHJ-6 (parent), Mutiara-1 (parent), the crossing of GHJ-7 and Mutiara-1, and the crossing of Mutiara-1 and GHJ-6 have the same value to the rate of infection with the crossing of Mutiara-1 and GHJ-6 (r=lowest) but show the light disease severity which can be considered being selected as a source of resistant genes. Another indicator that can be used to evaluate this eligibility of genotype as a source of resistant genes is a decrease in crop yields due to soybean leaf rust infections which still need more detail

examinations.

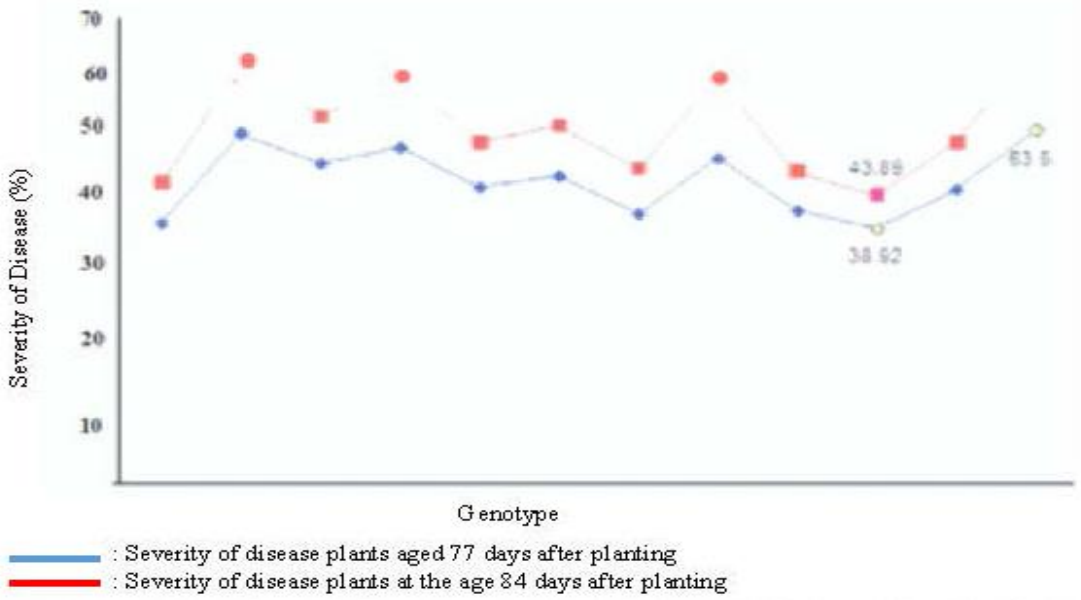

Figure 2. Graph of the relationship between increasing of the leaf rust disease on soybean genotype with different plant age

Severity of disease of 12 soybean genotypes can be divided as follow: four-and eight genotypes parent result of a cross between parent, were significantly different between genotypes ranged from 38.92 to 53.23 percent, with the rate of infection r = 0.02 to 0.03 units/day. Genotype resulted from crossing between Mutiara-1 with GHJ-7 has the lightest KP = 38.92 per cent and the lowest infection rate (r = 0.02 units/day) indicates the degree of resilience category as resistant, so it is considered the most worthy to be chosen as sources of resistance genes for assembling resistant varieties. The degree of resilience of the eleven other genotypes (genotypes four parent and seven genotypes result of crossing between parent) categorized as somewhat resistant.

Among the eleven genotypes with a degree of resilience rather resistant which showed the value of KP, including the infection rate is equal to the genotype of crossbred Mutiara-1 with GHJ-7 may also be considered viable as a source of the resistance gene, but still need to pay attention to the magnitude of crops due soybean leaf rust infection as an indicator and for it is necessary to test specifically in more detail. Genotypes with a degree of resilience rather resistant which has KP include lightweight with r equal to the result of crossing the Mutiara-1 with GHJ-7 there are four genotypes i.e. GHJ-6, Mutiara-1, the result of crosses between GHJ-7 with Mutiara-1 and the result of crosses between Mutiara -1 with GHJ-6.

CONCLUSION

Based on IWGSR rating codes, from 12 tested genotypes (four genotypes parents and eight genotypes resulted from crossing between these), only one genotype resulted from crossing between Mutiara-1 and GHJ-6 was categorized resistant. In addition to resistance, this genotype is the lightest severity of disease (KP) and the lowest infection rate (r), thus it can be said that it is the most eligible source of resistant genes to be selected for the breeding of resistant varieties

CONFLICT OF INTEREST

This study was conducted without any conflict of interest**.**

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AUTHOR CONTRIBUTIONS

Moh. Setyo Poerwoko designed his plant breeding program as a whole, Endang Budi Tri Susilowati designed leaf rust disease, Moh. Rizal Pahlevi designed the infection rate of soybean leaf rust

disease.

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