Evaluation of Resistance Soybean (*Glycine max*) Germplasm Accession to Rust Disease (*Phakopsora pachyrhizi*)

Sumartini1* and Suhartina1

1Indonesian Legumes and Tuber Crops Research Institute, Jl Raya Kendalpayak Km 8, Malang 65101, East Java, Indonesia.

*E-mail: sumartiniputut@yahoo.co.id

Abstract. Rust disease is an important disease on soybean. It caused yield losses, up to 85%. The best control measured was planting the resistant varieties. The resistant gens can be obtain from the germplasm collection. Evaluation of the resistant soybean germplasm collection infected rust disease was done at Kendalpayak Experimental Station, Indonesian Legumes and Tuber Crops Research Institute (ILETRI), from June to September 2013, with 225 genotypes of soybean germplasm collection. Besides, at green house of Plant Protection Unit in ILETRI, from March to May 2014, with 50 genotypes that have already screened in the field. Observation of rust disease resistance based on the International Working Group on Soybean Rust system. Out of 225 soybean genotypes tested there was not any resistant one, 218 genotypes were moderate resistant, and 7 genotypes were moderate susceptible to rust disease in the field. There was not any resistant one, 8 genotypes were moderate resistant, 33 genotypes were moderate susceptible, and 9 genotypes were susceptible to rust disease in the green house. Eight soybeans accession had consistent result in the green house and the field, there were MLG 0852, MLG 0887, MLG 937, MLG 1024, MLG 1034, MLG 1035, MLG 1065, MLG 1066.

Keywords: soybean, *Phakopsora pachyrhizi*, screening, germplasm collection

1. Introduction

Soybean is an important food Crop in the world, included in Indonesia. From soybean can be made of tofu, tempeh, soy sauce, or soymilk. Taking the fourth of foods, it can be made various foods product as source of protein Indonesian people. Indonesian goverment has a programme called “swasembada kedelai” means increasing soybean production to provide soybean needed. Increasing soybean production have some constrains, one of them was rust disease infection. Rust disease was so call “Asian soybean rust”. It was caused by the fungus *Phakopsora pachyrhizi* [1]. In Indonesia, the rust disease is an important disease in soybean crops in the dry season. Infected leaves caused yellowing, dry, and fall before pod mature [2].

It was widely spread in the area of soybean production centres in the world and results in significant yield loss. Distribution of rust disease started from Japan and East Asia in 1902, go to Southeast Asia (Indonesia) and Australia in 1914, while in Asia has reached India in 1950, and to Hawaii in 1994, then to South Africa (1920) and has reached Uganda in 1996. in the years 2001 to 2002 rust disease occurs in South America, and in 2004 has spread to the north to the United States [3]. In Indonesia, the first time occurred in Yogyakarta and Surakarta, reported by Raciborski in 1900,
at that time the caused fungus was mention as *Uromyces phaseoli*, and more recent intensive observations made in 1960's.

Yield losses can reach 90% in Indonesia [4], 10-40% on local varieties in Thailand, 23-50% in Taiwan [5], 80% in Nigeria [6], and 85% in the USA if the environment is suitable for the development of the disease [7]. Based on the symptom there were two types, reddish brown (RB) type, and TAN types, RB was more virulent than TAN [8]. Hartman et al. [7] stated that there are four types of genes responsible for controlling rust disease resistance in soybean is Rpp1, Rpp2, Rpp3, and Rpp4. Resistant of rust disease was also control by Rpp5 gen that is a resesif one [9].

One of control measured rust disease on soybean is planting the resistant varieties. Resistant variety was important component in integrated disease management [10]. The resistant of rust disease was one of the requirements in realese a new variety. Indonesian Legumes and Tuber Crops Research Institute have gene bank of soybean, it was consisted in some difference genetic, it was so call germplasm collection. The collection were more than 3000 accession of soybean. Source of resistant gene to rust disease could be known from the germplasm collection. Besides, the resistant to rust disease is durable resistance. The fungus could be form a new race that is more virulent, therefore the formation of superior resistant soybean to rust disease is still needed. The objective of the study is to evaluate the resistance of soybean germplasm collection to rust diseases.

2. Materials and methods

2.1. Field experiment

The experiment was conducted at Kendalpayak Experimental Station, Indonesian Legumes and Tuber Crops Institute (ILETRI), in the dry season I, 2013 (June-September 2013). Materials to be tested were 225 soybean genotypes from the germplasm collection. Soybean seeds of each genotype were planted in plots with a plot size of 2 m x 1 m, and the spacing of 50 cm x 10 cm (four lines, 10 planting holes/line), and two plants / hole. Observations were done to dry seed weight and the weight of 100 seeds.

2.2. Green house experiment

The experiment was conducted at the emplacement of green house of Plant Protection Unit in ILETRI, from March to May 2014. Material used were 50 genotypes of soybean germplasm collection that were moderately resistant and high yield in the field (>100 gr/2m²). Soybean seeds of selected genotype were planted in polybags, 5 polybags/genotypes, and two plants / hole.

2.3. Inoculation

Four weeks old plants were inoculated with rust disease. Spore suspension (spore density of $10^4$ spores/ml) was originated of rust-infected leaved from inoculums plantation source. The day before inoculation infected leaves were taken to be incubate at 100% humidity condition in the laboratory for 24 hours. After incubation the spores were taken by using a brush, and then the spore were diluted into water. The spore suspension was homogenized by using Tween-20 one drop per litre. Inoculation is done by spraying a suspension of spores to the leaves in the afternoon, the plastic covered the hole plants just after inoculation to make high humidity during a night. To avoid pest attacking, insecticides (carbofuran, sipemetrin, cyhalothrin) was applied when needed.

2.4. Rust disease assesment

Rust disease resistance was observed on three sample plants in the field, and 3 pots in the the green house three times at 7, 8, and 9 weeks after planting followed by the method of International Working Group on Soybean Rust [11] as:

- The position of plant diseases: 1 = 1/3 lower plant, 2 = 2/3 middle part of plant , 3 = 1/3 crop top
- Severity of disease: 1 = no pustules 2 = mild (1-8 pustules / cm²) 3 = moderate (9-16 pustules / cm²) 4 = severe (> 16 pustules / cm²)
• Status / presence of spores in the pustules: 1 = no pustules, 2 = no spores in pustules, 3 = pustules with spore.
The resistance was determined by the following criteria:
Immune: Scores 111
Resistant: Score 122, 123, 132, 133, 222, 223
Moderately resistant: Score 142, 143, 232, 233, 242, 243, 322, 323
Moderately susceptible: Score 332, 333
Susceptible: Scores 343

3. Results

3.1. Field experiment
Incubation period (time from inoculation up to appeared the symptom) in the field was 10 – 14 days, in this case, the symptom was appeared at 10 days after inoculation. The longer period of disease infection, the more severe rust infected the plant and the higher intensity of the disease, and cause immature defoliation. The result of the third observation was shown in table 1. Most of the soybean accessions were moderately resistant, means that there have the same ancestor. The category of rust disease resistance consisted in 3 digit. The first digit was position of the disease. The disease developed from the lowest part of the leaves that near soil to the upper part of the leaves. Nine weeks after planting, the position of rust disease of all genotypes tested were in score 3. It’s means that rust disease has already reached on top area of the plant. The second digit was disease intensity. It were almost all of the genotypes tested have 1 – 8 pustules/cm², or 94%, and the rest of genotypes were have 9 – 16 pustules/cm², or 6%. The score of spore status was 3 means that their some pustules was already mature and come out of spores, it was vary from 1.00 up to 26.33 pustules. The pustule was a tissue of the fungus that contained a lot of spores, when the spore released from the pustules and come in to the leaves, it will form a germtubes and start to infect the leaves. The third digits, the existence of the spore 2.00 – 3.00 means there are some spore come out the pustules, a part of the pustules was already opened, and ready to release the spore.

3.2. Green house experiment.
Fifty genotypes that classified as moderately resistant and have high yield selected to be tested. The result of the research was shwon in table 2. A disease will occur if there are three interacting factors namely, host plant, pathogen, and environmental factor particularly temperature, humidity, and light. Rust disease well-developed at 15-28°C, >90% humidity, and <700 lux light radiation [12]. In field, study was conducted from June to August, when the average temperature was 30°C, hence the intensity of rust disease at the observation time is not high. On the other hand, stress of rust disease in greenhouse is very high due to well-developing rust disease. Based on the number of pustules, among soybean germplasm tested, their have different number of pustules/cm², the number of pustules 1-8 pustules/cm² occupied 64%, 9 – 16 pustules/cm² was 18%, and >16 pustules/cm² was 18%, where it did not any laters classified in the test field. There is a true negative correlation between pustule number and the seed weight ($r^2 = -0.485$) means that the more number of pustule, the less weight of seed.

The yield were classified into four categories, there were more than 300 gr/2m² were 2%, 200 – 300 gr/2m² were 14%, 100 – 200 gr/2m² were 68%, and less than 100 gr/2m² were 16%. Besides, the seed size were classified into three, there were small (less than 10 gr/100 seeds) occupied 78%, medium (10 – 12 gr/100 seeds) occupied 18%, and big ( more than 13 gr/100 seeds ) occupied 4%.

4. Discussion
The result of another study also were not found the resistance genotypes, similar result showed that out of 10 genotypes, there were nine genotypes were moderately resistance and one genotype was susceptible to rust disease [13]. Besides, out of 28 genotypes tested most genotypes were susceptible
to moderately resistant, and they found seven genotypes were resistance [14]. It was rather difficult to find resistance genotypes. It may be caused of only few source of resistant genotype. The resistant genotype can be achieved from “centre of origin of soybean”, in this case was China country. Some genes that responsible to resistant could be inserted to other soybean plant with special modern method.

Table 1. Score and categories of soybean accession in the field.

| Score IWGRS | Category | Soybean Accession |
|-------------|----------|-------------------|
| 332         | MS       | MLG 0101, MLG 0132, MLG 0379, MLG 0465, MLG 0591, MLG 0713, MLG 0771 |
| 322         | MR       | MLG 0001, MLG 0010, MLG 0014, MLG 0015, MLG 0027, MLG 0030, MLG 0040, MLG 0042, MLG 0054, MLG 0057, MLG 0070, MLG 0071, MLG 0078, MLG 0080, MLG 0081, MLG 0086, MLG 0088, MLG 0089, MLG 0104, MLG 0106, MLG 0110, MLG 0113, MLG 0118, MLG 0121 |
| 333         | MR       | MLG 0005, MLG 0006, MLG 0041, MLG 0084, MLG 0086, MLG 0088, MLG 0089, MLG 0092, MLG 0096, MLG 0127, MLG 0144, MLG 0147, MLG 0152, MLG 0167, MLG 0171, MLG 0185, MLG 0187, MLG 0195, MLG 0215, MLG 0228, MLG 0229, MLG 0231, MLG 0234, MLG 0242, MLG 0253, MLG 0255, MLG 0288, MLG 0293, MLG 0293, MLG 0307, MLG 0313, MLG 0317, MLG 0320, MLG 0331, MLG 0336, MLG 0338, MLG 0343, MLG 0345, MLG 0379, MLG 0383, MLG 0386, MLG 0392, MLG 0393, MLG 0395, MLG 0417, MLG 0439, MLG 0464, MLG 0466, MLG 0470, MLG 0471, MLG 0474, MLG 0476, MLG 0479, MLG 0480, MLG 0486, MLG 0490, MLG 0502, MLG 0525, MLG 0528, MLG 0533, MLG 0551, MLG 0566, MLG 0570, MLG 0571, MLG 0572, MLG 0576, MLG 0610, MLG 0614, MLG 0618, MLG 0621, MLG 0637, MLG 0639, MLG 0645, MLG 0654, MLG 0663, MLG 0669, MLG 0687, MLG 0689, MLG 0696, MLG 0706, MLG 0710, MLG 0711, MLG 0712, MLG 0718, MLG 0720, MLG 0738, MLG 0739, MLG 0753, MLG 0758, MLG 0764, MLG 0767, MLG 0768, MLG 0770, MLG 0779, MLG 0780, MLG 0781, MLG 0782, MLG 0795, MLG 0796, MLG 0799, MLG 0801, MLG 0804, MLG 0813, MLG 0821, MLG 0826, MLG 0829, MLG 0830, MLG 0831, MLG 0832, MLG 0835, MLG 0836, MLG 0838, MLG 0842, MLG 0848, MLG 0850, MLG 0852, MLG 0862, MLG 0887, MLG 0900, MLG 0903, MLG 0908, MLG 0916, MLG 0918, MLG 0919, MLG 0924, MLG 0925, MLG 0929, MLG 0931, MLG 0932, MLG 0933, MLG 0937, MLG 0941, MLG 0942, MLG 0954, MLG 0955, MLG 0961, MLG 0972, MLG 0974, MLG 0984, MLG 1000, MLG 1010, MLG 1016, MLG 1020, MLG 1024, MLG 1027, MLG 1029, MLG 1033, MLG 1034, MLG 1035, MLG 1036, MLG 1041, MLG 1050, MLG 1053, MLG 1054, MLG 1058, MLG 1059, MLG 1061, MLG 1062, MLG 1063, MLG 1064, MLG 1065, MLG 1066, MLG 1067, MLG 1068, MLG 1069, MLG 1070, MLG 1071, MLG 1073, MLG 1075, MLG 1076, MLG 1077, MLG 1078, MLG 1079, MLG 1080, MLG 1081, MLG 1082, MLG 1083, MLG 1085, MLG 1086, MLG 1087, MLG 1088, MLG 1089, MLG 1090, MLG 1091, MLG 1092, MLG 1093, MLG 1094, MLG 1095, MLG 1096, MLG 1097, MLG 1098, MLG 1099, MLG 1100, MLG 1101, MLG 1102, MLG 1103 |

This result is similar to the screened result of peanut germplasm accession against rust diseases [15]. Among soybean accession tested, the yield range of 100 - 200 gr/2m² has dominant one, equivalent 0,5 – 1,0 ton/ha, it were 68%, the yield range of 200 – 300 gr/2m², equivalent 1,0 – 1,5 ton/ha, occupaid 14%, and the yield range of more than 300 gr/2m² occupied only 2%. The size of soybean seed were categorized into three, as small, medium, and big seed, there were 78%, 18%, and 4% respectively. Small seed has dominant one it can usually be use for Indonesian “tempe”.

Eight soybeans accession that classified as moderate resistant to rust disease had consistently result in the field and the green house there were MLG 0852, MLG 0887, MLG 937, MLG 1024, MLG 1034, MLG 1035, MLG 1065, MLG 1066 (table 3). These soybean accession also had higher yield, it could called tolerant genotype, and could be source of breeding lines for the future time.
Table 2. Resistance of soybean germplasm collection to rust disease. Greenhouse of Indonesian Legumes and Tuber Crops Research Institute. Dry Season 2014.

| No | Accession number | Rust disease position | Pustule number | Spore status | Disease score | Resistance criteria |
|----|------------------|-----------------------|----------------|--------------|---------------|---------------------|
| 1  | MLG 0005         | 3                     | 18             | 3            | 343           | S                   |
| 2  | MLG 0014         | 3                     | 13             | 2            | 333           | MS                  |
| 3  | MLG 0078         | 3                     | 12             | 2            | 333           | MS                  |
| 4  | MLG 0081         | 3                     | 15             | 2            | 333           | MS                  |
| 5  | MLG 0144         | 3                     | 14             | 2            | 333           | MS                  |
| 6  | MLG 0253         | 3                     | 25             | 3            | 343           | S                   |
| 7  | MLG 0293         | 3                     | 13             | 2            | 333           | MS                  |
| 8  | MLG 0313         | 3                     | 13             | 2            | 333           | MS                  |
| 9  | MLG 0320         | 3                     | 13             | 2            | 333           | MS                  |
| 10 | MLG 0345         | 3                     | 11             | 2            | 333           | MS                  |
| 11 | MLG 0465         | 3                     | 18             | 3            | 343           | S                   |
| 12 | MLG 0470         | 3                     | 19             | 3            | 343           | S                   |
| 13 | MLG 0551         | 3                     | 16             | 2            | 333           | MS                  |
| 14 | MLG 0621         | 3                     | 14             | 2            | 333           | MS                  |
| 15 | MLG 0663         | 3                     | 18             | 3            | 343           | S                   |
| 16 | MLG 0669         | 3                     | 15             | 2            | 333           | MS                  |
| 17 | MLG 0767         | 3                     | 15             | 2            | 333           | MS                  |
| 18 | MLG 0768         | 3                     | 12             | 2            | 333           | MS                  |
| 19 | MLG 0780         | 3                     | 13             | 2            | 333           | MS                  |
| 20 | MLG 0829         | 3                     | 13             | 2            | 333           | MS                  |
| 21 | MLG 0831         | 3                     | 15             | 2            | 333           | MS                  |
| 22 | MLG 0852         | 3                     | 7              | 2            | 323           | MR                  |
| 23 | MLG 0887         | 3                     | 7              | 2            | 323           | MR                  |
| 24 | MLG 0900         | 3                     | 11             | 2            | 333           | MS                  |
| 25 | MLG 0918         | 3                     | 15             | 2            | 333           | MS                  |
| 26 | MLG 0919         | 3                     | 19             | 3            | 343           | S                   |
| 27 | MLG 0924         | 3                     | 17             | 3            | 343           | S                   |
| 28 | MLG 0929         | 3                     | 13             | 2            | 333           | MS                  |
| 29 | MLG 0931         | 3                     | 11             | 2            | 333           | MS                  |
| 30 | MLG 0932         | 3                     | 12             | 2            | 333           | MS                  |
| 31 | MLG 0933         | 3                     | 11             | 2            | 333           | MS                  |
| 32 | MLG 0937         | 3                     | 7              | 2            | 323           | MR                  |
| 33 | MLG 0954         | 3                     | 17             | 2            | 343           | S                   |
| 34 | MLG 0955         | 3                     | 12             | 2            | 333           | MS                  |
| 35 | MLG 0972         | 3                     | 13             | 2            | 333           | MS                  |
| 36 | MLG 1010         | 3                     | 12             | 2            | 333           | MS                  |
| 37 | MLG 1016         | 3                     | 17             | 3            | 343           | S                   |
| 38 | MLG 1024         | 3                     | 7              | 2            | 323           | MR                  |
| 39 | MLG 1034         | 3                     | 7              | 2            | 323           | MR                  |
| No | Accession number | Rust disease position | Pustule number | Spore status | Disease score | Resistance criteria |
|----|------------------|-----------------------|---------------|-------------|--------------|-------------------|
| 40 | MLG 1035         | 3                     | 8             | 2           | 323          | MR                |
| 41 | MLG 1052         | 3                     | 14            | 2           | 333          | MS                |
| 42 | MLG 1053         | 3                     | 16            | 2           | 333          | MS                |
| 43 | MLG 1056         | 3                     | 11            | 3           | 333          | MS                |
| 44 | MLG 1062         | 3                     | 14            | 2           | 333          | MS                |
| 45 | MLG 1065         | 3                     | 7             | 2           | 323          | MR                |
| 46 | MLG 1066         | 3                     | 6             | 2           | 323          | MR                |
| 47 | MLG 1069         | 3                     | 10            | 2           | 333          | MS                |
| 48 | MLG 1070         | 3                     | 12            | 2           | 333          | MS                |
| 49 | MLG 1087         | 3                     | 11            | 2           | 333          | MS                |
| 50 | MLG 1092         | 3                     | 7             | 2           | 323          | MR                |

*S= Susceptible  
**MS= Moderately susceptible  
^cMR= Moderately resistance

Table 3. Superior soybean accession.

| No | Accession number | Rr^a in the field | Rr^b in the green house | Seed weight | Seed size |
|----|------------------|--------------------|-------------------------|-------------|-----------|
| 1. | MLG 0852         | MR                 | MR                      | 234,0       | 8,5       |
| 2. | MLG 0887         | MR                 | MR                      | 198,0       | 7,5       |
| 3. | MLG 0937         | MR                 | MR                      | 253,5       | 10,0      |
| 4. | MLG 1024         | MR                 | MR                      | 238,5       | 8,5       |
| 5. | MLG 1034         | MR                 | MR                      | 233,5       | 12,0      |
| 6. | MLG 1035         | MR                 | MR                      | 235,0       | 8,0       |
| 7. | MLG 1065         | MR                 | MR                      | 245,0       | 8,0       |
| 8. | MLG 1066         | MR                 | MR                      | 381,0       | 7,0       |

^aRr = Rust resistant  
bMR = Moderate Resistant

Report from Indonesia, with the same method to assess rust disease found that out of 47 breeder lines, non of them was resistant, ten genotypes were moderate resistant, 36 genotypes were moderate susceptible, and one genotype was susceptible [16]. In line with a study in 2017 that reported only 10 soybean genotypes that were moderately resistant to rust disease [17]. Other researcher found that all of genotypes (26 soybean genotypes) were moderate susceptible to rust disease, although six of them still had high yield, it was call tolerant variety [18].

Similar research reported, with 1 – 9 scale for assessment the rust disease on soybean germplasm accession found that from 84 genotypes, non of the genotypes showed immune reaction to rust disease, two genotypes were resistant (EC 241778 and EC 241780), and the remained were highly susceptible to rust disease [19].

Iwo [13] stated that by using simpler method based on the intensity of rust disease in 1-5 scale. Through this method, it classified the soybean genotype resistance to rust disease. Out of 28 tested genotypes, there were seven resistant genotypes and the dominant genes controlling resistance of rust disease were found in three different loci.

Paul [19] classified soybean resistance against rust disease into two groups with four types of fungus isolates Phakospora pachyrhizi and symptom expressed by reddish-brown (RB) and TAN. The 34 tested genotypes comprised of 28 sporulation TAN type-genotypes and six less spores RB type-
genotypes. In Indonesia, RB symptom are more dominant than TAN while strains of P. pachyrhizi has been no report.

The resistance of plant to disease could be occurred by morphologic or biochemist. For example a plant with contained high phytoalexin. It was derevide of phenolic and flavonoid that were antimicrobial. The phenolic and flavonoid inside of soybean seed has already reported, but they were not combined between these antimicrobial with rust disease intensity [20].

5. Conclusion
In the field, out of 225 soybean genotypes tested there was not any resistant one, 218 genotypes were moderately resistant, and 7 genotypes were moderately susceptible. In the green house, out of 50 selected genotypes tested there was not any resistant one, 8 genotypes were moderately resistant, 33 genotypes were moderately susceptible, and 9 genotypes were susceptible to rust disease. Eight soybeans accession had moderate resistant consistently result in the green house and the field, there were MLG 0852, MLG 0887, MLG 937, MLG 1024, MLG 1034, MLG 1035, MLG 1065, MLG 1066.

References
[1] Semangun H 2004 Diseases of Food Crops in Indonesia (Yogyakarta: Gajah Mada University Press) 475 pp.
[2] Kumidini S, Godoy C V, Board J E, Omielan J and Tollenaar M 2008 Crop Scince 6(48) p 2334 – 2342.
[3] Miles RM, Frederick R D and Hartman G L 2003 Soybean Rust: Is The US Soybean Crop at Risk? [http://www.apsnet.org online/feature/rust/]
[4] Sudjono M S, Amir M M and Roechan M 1985 Rust disease and their control In Sadikin Somaatmadja et al (ed) Soybean Food Crops Research Centre Institute. Bogor p 331-356.
[5] Backman P A, Sinclair J B and Sinclairia J B 1989 Compendium of Soybean Diseases The American Phytopathological Society USA 69 p.
[6] Twizeyimana M, Ojiangbo P S, Icotun T, Ladipo J L, Hartman G L and Bandiopahayay R 2008 Plant Disease 92 p 947 – 952.
[7] Hartmant G L, Miles M R and Frederick R D 2005 Plant Disease 89(6) p 664-666.
[8] Garcia A, Calvo E S, de Souza Kuhl, Harada R A, Hiromoto D M and Viera L G E 2008 Theor Appl Genet 117 p 545-553.
[9] Hartman G L, Hill C B, Twizeyimana M, Miles M R and Bandypadhyay R 2011 Interaction of soybean and Phakopsora pachyrhizi the cause of soybean rust CAB Review Perspective in Agriculture Veterinary Science Nutrition and Natural Resources http://www.cabi.org/cabreview
[10] Shanmugasundaram 1977 The International working group on soybean rust and Its proposed soybean rust rating system Work shop on rust of soybean The problem and research needs Manila Philippines 28 Feb – 4 March 1977.
[11] Sumartini and Kuswanto H 2014 Resistance Evaluation of Large Seeded Soybean Lines to Rust Disease Proceeding of the 4th Annual Basic Science International Conference (Basic) 2014 Volume 4 p 1 – 4.
[12] Sumartini and Trustinah 2013 Evaluation of resistance on peanut germplasm accession to rust disease (Puccinia arachidis) Proceeding of Indonesian Breeder Society Congress Bogor Desember 2013.
[13] Iwo G A, Ittah M A, Osai E O 2012 Journal of Agricultural Science 4(10) Canadian Centre of Science and Education.
[14] Sudjono M S 1984 Epidemiology and Control measured of rust disease on soybean [Dissertation] (Bogor: Bogor Agriculture University) 151 pp.
[15] Inayati A, Eriyanto Y and Hariatim 2015 Evaluation of promoting tropic soybean lines to rust disease p 408 - 417 In Kasno A, Adie M M, Rahmiana A A, Heriyanto, Suharsono, Yusnawan E, Tastra I K, Ginting E, Iswanto R, Harnowo D (eds). Proceeding Seminar of
Legumes and Tuber Crops Research

[16] Hardaningsih S and Hadi M 2014 Reaction of promoting soybean lines to rust disease and downy mildew. p 264 – 270. In Saleh N, Harsono A, Nugrahaeni N, Rahmiana A A, Sholihin, Yusuf M, Heriyanto, Tastra I K, Adie M M, Hermanto, Harnowo D (eds). Proceeding Seminar of Legumes and Tuber Crops Research.

[17] Sulistyo A and Sumartini 2017 Biodiversitas 17(1) 124-128

[18] Khan M H, Tyagi S D and Dar Z A 2013 Screening of soybean genotypes resistance to rust disease, Yellow Mosaic Virus and pod shattering. In Soybean Pest Resistance. http://www.dx.doi.org/10.5772.54697.

[19] Paul C, Hill C B and Hartman G L 2011 Plant Disease 95 p 1007-1012

[20] Yusnawan E, Inayati A and Kuswantoro H 2015 Total phenolic contents and antioxidant activities of ten soybean promoting lines tolerant to acid soil Proceeding Seminar of Challenges microbial technologies for Foods and Health, November 2014, (Solo: Indonesia)