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Resistance level of several soybean lines of M_6 generation to stem rot disease *Athelia rolfsii*

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Abstract. *Hanafiah DS, Safni I, Siregar LAM, Famanik RIM, Lestmi A, Matondang M. 2020. Resistance level of several soybean lines of M6 generation to stem rot disease* Athelia rolfsii. *Biodiversitas 21: 4537-4542. Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. is a soil-borne pathogen that causes stem rot disease on plants. The attack is one of the causes of low soybean productivity hence induction of plant resistance is needed to overcome these problems. Mutagenesis is one of the breeding techniques for inducing genetic variability especially for disease-resistant characters in soybean. This research aimed to obtain selected soybean plant lines (*Glycine max* L. Merr) of M_6 generation based on resistant character against stem rot disease *A. rolfsii*. This research was conducted at the Laboratory of Plant Disease and research field of Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia and soybean planting was carried out on agricultural field, Universitas Sumatera Utara from March to September 2018. This research used augmented design. The treatments were 8 mutant genotypes derived from radiation mutagenesis, that is; Anjasmoro, Argomulyo, and Kipas Putih varieties. The results showed that the analysis of resistant levels on M_6 generation based on disease incidence resulted in 2 lines with resistant criteria to stem rot disease *A. rolfsii*, that is; M100A25 (5/3) and M200A11 (32/3). Observation of intensity of disease attacks showed that Anjasmoro, Argomulyo, M100A6 (31/1), and M200A12 (6/5) had the lowest resistance level compared to other genotypes. The mutant lines that had high category level of resistance are candidates for resistance lines against stem rot disease *A. rolfsii*.

Keywords: *Athelia rolfsii*, M_6 generation, resistant, soybean, stem rot disease

INTRODUCTION

Global warming has changed global, regional, and local climate conditions. One of the effects of global climate change is an increasing population of pests and plant diseases (Zayan 2019). Soybean plants in tropical countries, including Indonesia, face challenges from various potential pests and diseases. Increasing the earth's temperature will increase soybean pest and disease population as well (Susanti et al. 2018). The main effect of state, year, pre- and post-discovery of soybean rust, region, and zones based on year, harvest area, and production, were significant on total economic loss as a function of diseases (Bandara et al. 2020). One of the barriers that can reduce soybean production is a disease caused by a fungus attack *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. (formerly *Sclerotium rolfsii* Sacc.). The infected plants will die and pathogens can survive for long time in the soil in the form of *sclerotia* (Peltier et al. 2012).

Athelia rolfsii can cause stem rot disease in bean plants. Humid environmental conditions also cause this fungus to infect stems and leaves of bean plant near the soil surface and can be a bridge to the spreading of mycelium growth in other parts of the plant (Pudjihartati et al. 2006). Seedborne pathogenic fungi in beans used for seed reduce germination, emergence, growth, and yield, whereas in beans used for food they can reduce the nutritional value or produce toxins making the beans unsuitable for consumption (Marcenaro and Valkonen 2016). Husna (2016) stated that Anjasmoro, Willis, and Grobogan varieties do not show resistance to pathogens that cause stem rot disease. The highest percentage of disease incidence was found in the Anjasmoro variety at 55.56%, followed by Willis at 44.44% and Grobogan at 33.33%. Globally, *A. rolfsii* can cause yield reductions as high as 60% (Cunha et al. 2010).

Various methods to control this disease have been carried out but they have not succeeded in reducing the attack of soil-borne diseases in bean plants. Chemical fungicides are effective in suppressing soil-borne fungi. Even though they are available with a variety of trademarks and active ingredients, but they are less applicable to bean plant farmers in developing countries like Indonesia due to the high price of pesticides (Tantawizal and Rahayu 2017).

The use of disease-resistant varieties is one of the practical control methods that can prevent or be preventive. Therefore, various studies have been carried out to produce resistant plants against soil-borne fungal diseases such as stem rot disease (Sumartini 2012). One strategy to create disease-resistant plants against stem root disease is using mutation breeding techniques. Mutation is a sudden heritable change in an organism and generally induces structural and composition changes in genome, chromosome, gene, or DNA (Dhanavel et al. 2012).

A mutation breeding study related to the Anjasmoro; soybean variety plant was conducted in M_1 generation (Sibarani et al. 2014), M_2 generation, M_3 generation (Sihombing et al. 2016), and the results showed that 300 Gy irradiation dose decreased the growth and productivity of plant population, and 100 Gy dose increased the weight seeds per plant. M_4 generation was selected based on early maturing and high-producing characters (Bangun 2016).

The research conducted by Rahmah et al (2018), selected soybean lines of M_5 generation based on production character and resistance of stem rot disease. The results showed that the appearance of soybean agronomic character at fungal inoculation treatment was lower than the treatment without inoculation. Selection using *A. rolfsii* disease resulted in the putative selected mutant lines resistant to the stem rot disease, which was more commonly produced from populations of 100 Gy and 200 Gy. This study aimed to identify the stability of putative mutant lines at M_6 generation which then provide useful information in soybean improvement and breeding.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Plant Disease and research field of Faculty of Agriculture, University of Sumatera Utara, Medan, Indonesia. This research was conducted from March 2018 to September 2018.

Culture and production of *Athelia rolfsii*

The inoculum of *A. rolfsii* was collected from infected soybean plants in the field of Balai Benih Induk Sumatera Utara, Medan. The infected stem was cleaned with alcohol 90% for surface sterilization, and then rinsed three times with sterile water. The *A. rolfsii* was inoculated on the plant stem tissue and incubated at room temperature with the humidity around 58 % for ± 10-14 days. The growing *A. rolfsii* mycelium then isolated again to obtain pure cultures. The pure cultures were propagated into a Petridis containing PDA (Potato Dextrose Agar) media.

The PDA medium consists of 250 g potatoes, 10 g agar, and 10 g dextrose in 1 L of dH_2O. Potatoes were peeled, diced, and boiled with 1 liter of distilled water until cooked. After that, the potato extract water was mixed with agar and dextrose while stirring on the stove until it boiled. PDA media solution was put into the Erlenmeyer flask, and then autoclaved for 15 minutes with a temperature of 121ºC and pressure of 1 atm. The pure culture was inoculated into the Petridis containing PDA media and then incubated for 10-14 days. Pure culture characteristics are the formation of circular white fungus mycelia on the Petri dish.

To grow the fungi, dried corn kernels were used and soaked into water for 4 days. Then the corn kernels were put into a plastic polypropylene bottle (PP Bottle), and tied for 30 minutes. The corn kernel media was then autoclaved for 15 minutes at a temperature of 121ºC, with the pressure of 1 atm. This procedure referred to Hoa et al. (2015). The autoclaved media was cooled and inoculated with pure culture using 3 to 4 cork-borers from the pure culture of fungus. The inoculation was carried out in the Laminar Air Flow, and then the plastic bottle was covered with cotton coated with aluminum foil and clung-warp to avoid contamination. The substrate was incubated for 10 to 14 days at 28ºC temperature.

Seed selecting and planting

M_6 generation soybean seeds derived from the M_5 generation selection results were used in the experiment. There were 8 groups of lines namely M100A25 (5/3), M200A11 (32/3), M100A6 (31/1), M200A17 (18/5), M200A17 (13/6), M200A12 (6/5), M300A8 (35/7), and M300A6 (33/8). The control plants were Argomulyo, Anjasmoro, and Kipas putih varieties. Soybean seeds were planted on land plots with a size of 2.5 m x 13 m for optimal land and *A. rolfsii* inoculation land. The planting-hole was made using the tip of a finger, with a plant spacing of 30 cm x 15 cm. Each planting-hole was planted one soybean seed and then covered with topsoil. The plants were fertilized for 7 days after planting in accordance with the recommended dosage of soybean fertilizer needs, that is; Urea 2.3 g/planting hole, SP-36 3.4 g/planting hole, and KCl 4.5 g/planting hole by placing the fertilizer around the hole of the plant with a distance of 7-10 cm.

Inoculation of *Athelia rolfsii* fungi

According to Husna (2016), the application of inoculants to soybean plants is done by sowing corn substrate media containing *A. rolfsii* inoculant 15 g/plant around and near the base of the stems of 2 weeks-soybean plants.

Disease Incidence (DI)

Disease incidence observation of A. rolfsii was carried out at 3 days intervals within 8 times observations in the morning started from one week after the application of A. rolfsii (Tindaon 2008). According to Maden and Hughes (1999) and also Gashaw et al (2014), the incidence of disease and the intensity of the disease attack are determined by equation 1 and equation 2:

$$DI = \frac{n}{N} \times 100\% \quad (1)$$

Where:
n : Number of infected plants
N : Total number of plants

Disease attack intensity

The intensity of the disease attack was calculated by equation 2:

$$I = \frac{\Sigma (ni \times vi)}{N \times Z} \times 100\% \quad (2)$$

Where:
I : Disease Attack Intensity
ni : Number of plants infected in each attack category
vi : Scale value of each attack category
Z : The highest scale value
N : Number of plants observed

According to Yusnita et al (2005), the value of the scale for each category of attack on each plant is determined based on the symptoms of the attack caused by plants (Table 1).

Based on the percentage value of the disease attack intensity, all tested genotypes were placed in the following three categories (Table 2):

Table 1. The scale attacks of fungus *Athelia rolfsii*

Scale	Attack symptoms
0	Without attack
1	Necrosis with an area up to 0.5 stem circumference
2	Necrosis at 0.5-0.75 stem circumference
3	Necrosis has surrounded the stem, appeared brown spots and extended to the surface of the infected stem
4	Infected stems begin to droop and a number of leaves begin to wilt
5	Dead plants

Table 2. Categories of tested genotypes resistance level

Disease attack intensity (%)	Resistance level
0 – 20	High
≥ 20 – 30	Moderate
≥ 30	Low

RESULTS AND DISCUSSION

The disease incidence (DI) in the first observation interval (7 days after inoculation) determines the resistance of lines in the experiment (Figure 1). It was identified that the most susceptible line was M200A12 (6/5) with the DI value of 10.71%. On the other hand, the resistant lines included M100A25 (5/3), M200A11 (32/3), M200A17 (18/5), M300A8 (35/7), and M300A6 (33/8). This is consistent with the experiment of Amien and Carsono (2000), which stated that through radiation techniques, it can produce mutants or plants that have mutations with expected characters (Kim et al. 2004). Furthermore, gamma rays belong to ionizing radiation and radiation-induced ionizations may act directly on the cellular component molecules or indirectly on water molecules, causing water-derived radicals. These radicals can damage or modify important components of plant cells and they have been reported to affect the morphology, anatomy, biochemistry, and physiology of plants differently depending on the irradiation level.

The result of observation diagram of disease incidence at the 8th interval (28 days after inoculation) can be seen in Figure 2. The figure showed that the affected plant from plot 1 was M100A25 (5/3) by 8.93%, M200 A11 (32/3) by 7.14%, and Anjasmoro as the origin parent by 7.14%. The number of affected plant from plot 2 were M100A6 (31/1) by 14.21%, M300A8 (35/7) by 14.29%, and Kipas Putih variety by 14.29%. The number of affected plant from plot 3 was M200A17 (13/6) by 26.79%, M200A17 (18/5) by 12.50%, Kipas Putih variety by 14.29%, M200A12 (6/5) by 32.14%, and M300A6 (33/8) by 10.71%.

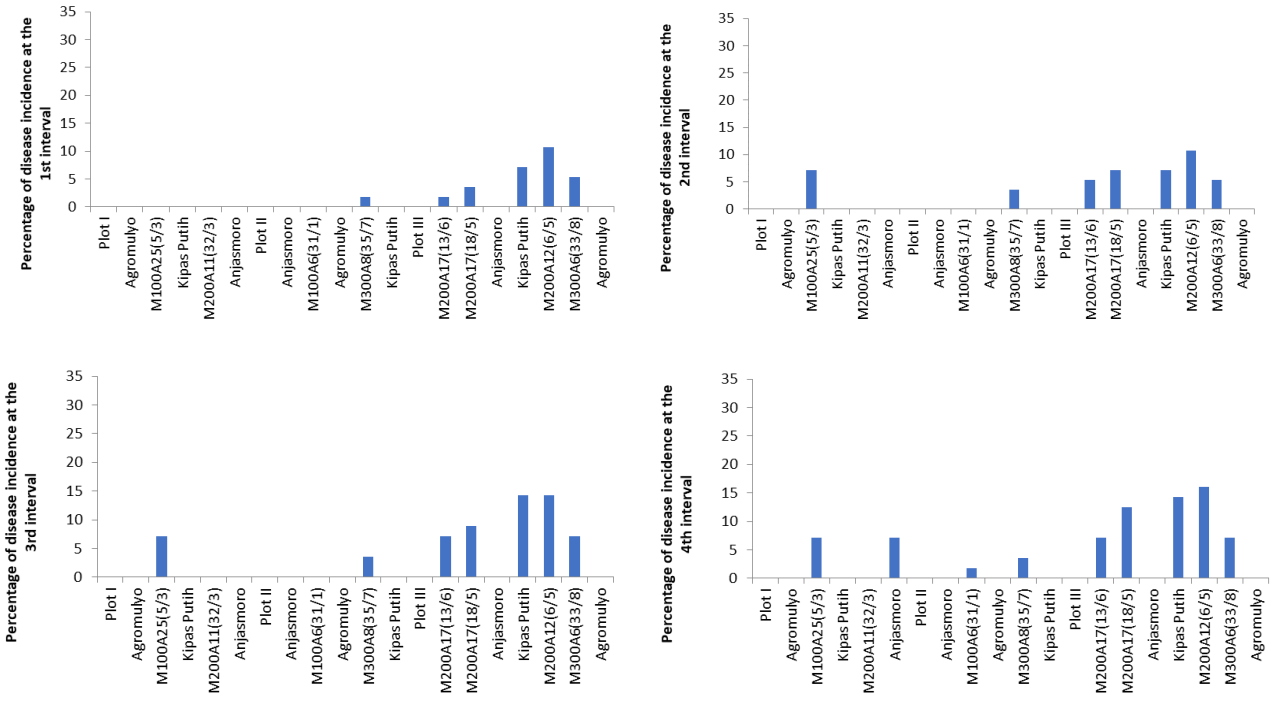

Figure 1. Percentage of disease incidence during the 1st – 4th interval (7-16 days after inoculation)

The result showed that there was genetic diversity due to mutation induction which could be seen through differences in the intensity of disease attacks in each mutation. According to Muduli and Misra (2007), induced mutation can rapidly create the variability of inherited traits in crops, both quantitatively and qualitatively. Post induced mutation has been effectively utilized in developing new and valuable alternations in plant characteristics that have contributed to increase yield potential or disease resistance (Gaswanto et al. 2016).

The research results showed that the spread of the disease during the 1st – 8th interval visually showed no symptoms of disease incidence significantly to the growth of the control plants (Anjasmoro as the original parent). The possible reason for this is because the diseases that are inoculated into the plants do not infect the plants optimally. This causes the disease incidence in Anjasmoro variety does not show significant differences compared to other lines. The environmental conditions at the time of the study were rainfall occurred at moderate intensity.

Observation was continued by dissecting the stem of the plant by observing the cross-section of the stem. The results showed that there were differences between the cross-sections of the stems infected by *Athelia rolfsii* compared with healthy stems (Figure 3). The tissue that was infected by the fungi was light brown and dark, but not watery. The symptoms of attack were also seen in which necrosis had surrounded the stem, appearing brown spots that have expanded on the infected stem. In addition, application and observation of disease were carried out during the rainy season, which results in eroded mycelium by rainwater. This was in accordance with the report of Sumartini (2012), which stated that the attack of soil-borne pathogens including *Athelia rolfsii* in plants begin with infection of the roots or stems bordering the surface of the soil and is characterized by the presence of mycelium which circles the stems until the plants wilt and dies.

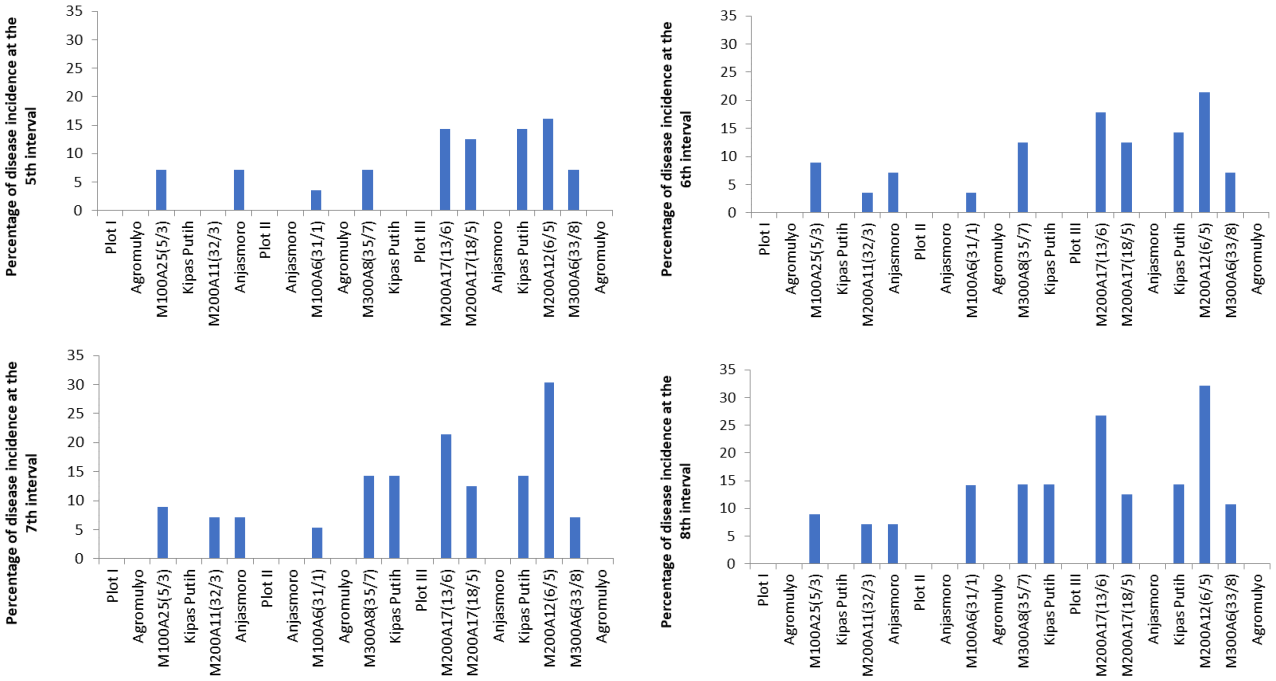

Figure 2. Percentage of disease incidence during the 5th – 8th interval (19-28 days after inoculation)

Figure 3. Longitudinal incision of stem soybean (A) soybean stems infected by *Athelia rolfsii*; (B) stems of healthy soybean plants

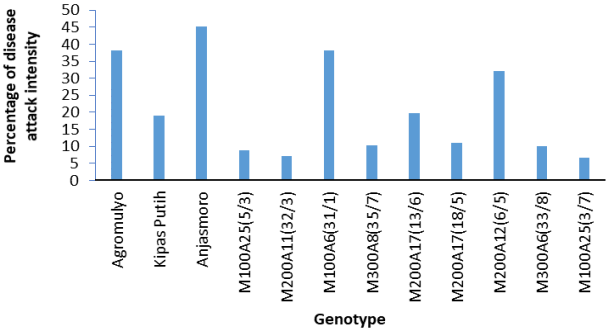

Figure 4. Disease attack intensity of M6 soybean generation lines on *Athelia rolfsii*

Additionally, seedlings were very susceptible and die faster once they became infected. Older plants that have formed woody tissue are gradually girdled by lesions and eventually die. Invaded tissues are pale brown in color and soft, but are not watery. The first symptom usually noticed by the homeowner or grower is wilt. Wilted plants often decline and die rapidly as a result of extensive lower stem rot (Kator et al. 2015). Furthermore, the first symptom of *Athelia rolfsii* infection is sudden wilting of a branch that is completely or partially in the contact with the soil. The junction of the branch with the stem near the soil level is the most favored point of attack then a white coating of fungus mycelium appears there. Sclerotia of mustard seed size appear gradually on the infected areas. Ranjan et al (2019) showed that initial symptoms of sclerotinia stem rot (SSR) began appearing on the main stem at 48 h post-inoculation, which spread as the disease progressed. By day 7^{th}, it was apparent that the resistance line had largely restricted fungal growth on the main stem and the red coloration observed at the site of infection had become more prominent, whereas the SSR infection had girdled the main stem of the susceptible line.

The intensity of the disease attacks (I) in Argomulyo variety was 38.10%, Kipas Putih variety was 19.05%, Anjasmoro variety was 45.24%, M100A25 (5/3) was 8.93%, M200A11 (32/3) was 7.14%, M100A6 (31/1) was 38.10%, M300A8 (35/7) was 10.36%, M200A17 (13/6) was 19.64%, M200A17 (18/5) was 11.07%, M200A12 (6/5) was 32.14%, and M300A6 (33/8) was 10.00% (Figure 4). The intensity of the stem root disease attack was depending on the genotypes and varieties of plants that are able to survive.

Observation of the disease attack intensity on the soil media that had been inoculated by the disease was carried out at harvest time. The disease spread in plants occurred from vegetative to generative periods. Germination experiment of *Abrus precatorius* seed had been shown that the majority of the imbibed seeds, in course of a day or two become covered by a profuse growth of fungal mycelia and ultimately fail to germinate (Ghosh et al. 2012).

Observations showed that the intensity of the attacks at Anjasmoro variety was higher than the intensity of the attack on all genotypes. The lowest intensity was found in the M200A11 (32/3) line at 7.14% and the highest intensity was found in M100A6 (31/1) at 38.10%. The differences in the intensity of this attack can be caused by genetic changes in individual plants that have been irradiated (mutations) and different radiation doses applied. The mutation rates are related to ionizing radiation to produce useful mutation rates in plants (Caplin and Willey 2018).

The intensity of *A. rolfsii*'s attack on Anjasmoro variety as the original parent was higher (45.24%) than the intensity of the attack on all genotypes tested. Observation of the disease attack intensity showed that Anjasmoro, Argomulyo, M100A6 (31/1), and M200A12 (6/5) had the lowest resistance level compared to other genotypes. The mutant lines that had high category level of resistance were candidates for resistance lines to stem rot disease *A. rolfsii*.

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