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Biochemical Resistance Mechanism of Several Genotype of Soybean to Rust Diseases Moh. Setyo Poerwoko, Endang Budi Trisusilowati dan Amarullah moh_setyo_poerwoko@yahoo.com endangbts@yahoo.com ABSTRACT The research to identify of the content of Fenol and Flavonoid compounds which set as phytoalexin of several soybean genotype from rust diseases. Four genotypes of soybean which were examined at Unej-1, Unej-2 (moderate resistance), Burangrang (tolerance) and Lokon (susceptible). Based on rating code of IWGSR Unej-1, Unej-2 and Burangrang were proved moderate resistance and Lokon which was considered susceptible reality showed moderate susceptible. At Unej-1, Unej 2 and Burangrang which moderate resistance showed an increase of amount of Fenol and Flavonoid compounds greater than Lokon which was moderate susceptible Key Words: Biochemical Resistance, Soybean and Rust diseases INTRODUCTION Soybean leaf rust disease caused by Phakopsora pachyrhizi Sydow is one of the causes of national soybean production degradation (Adisarwanto, 2005). According to Oka (1993) soybean yield losses due to leaf rust pathogen ranged from 20-80 percent, can even reach 100 percent on very susceptible varieties or when grown in monoculture and without crop rotation. The use of resistant varieties proved more effective in efforts to suppress soybean yield losses due to leaf rust disease and the control of a cheap, safe, and easy to implement (Adisarwanto, 2005). However, the resistance of a genotype is not absolute, but are subject to change, so there should always be an effort to create new and resistant varieties constantly. According Semangun (2001) the nature of the soybean plant resistance to leaf rust ranged from highly susceptible and highly resistant. The nature or mechanism of plant resistance to pathogens can be divided into mechanical resistance, biochemical and functional (Djaparuddin, 2000). According Sinaga (2003) Biochemical resistance is the resistance caused by the presence of certain chemical compounds that are toxic or inhibit the growth of pathogens. Groups of compounds are called phytoalexin (Salisbury and Ross, 1995). According Semangun (2001) is a phytoalexin compounds produced by host tissues in response to invading pathogens. Semangun (1996) reported that plants that are resistant or susceptible produce phytoalexin, but the plants are resistant formation faster and much more. And compounds which act as phytoalexin example glisolein in soybean roots, phenols and flavonoids in the vacuole and soybean leaf epidermal cells (Robinson, 1995), pisatin in pea pods, faseolin and fasiolidin in bean pods, ipomeamaron in sweet potato root, orchid bulbs orkinol in , and trifolirhizin in red clover roots (Salisbury and Ross, 1995), chitin in orange (Sinaga, 2003), and rishitin, lubimin and fitotuberin on potato (Semangun, 2001). Based on this phenomenon needs to be studied whether the mechanisms of soybean resistance to soybean leaf rust resistance biochemistry including content by comparing

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bean pods, ipomeamaron in sweet potato root, orchid bulbs orkinol in , and trifolirhizin in red clover roots (Salisbury and Ross, 1995), chitin in orange (Sinaga, 2003), and rishitin, lubimin and fitotuberin on potato (Semangun, 2001). Based on this phenomenon needs to be studied whether the mechanisms of soybean resistance to soybean leaf rust resistance biochemistry including content by comparing the presence of chemical compounds which act as phytoalexin, which is produced by soybean genotypes with different degrees of resistance. The study aimed to compare the content of chemical compounds responsible for the resistance of soybean genotypes to soybean leaf rust on susceptible genotypes, moderately resistant and tolerant. METHODS Biochemical mechanisms of soybean resistance to soybean leaf rust were tested in this study by comparing the genotypes of soybean were moderately resistant and tolerant to soybean leaf rust susceptible soybean genotypes. Testing is done by planting genotypes were tested in field trials Polytechnic of Jember in April to September 2006 soybean leaf rust pathogen inoculation is done naturally. Lokon genotypes tested were classified as susceptible, Unej Unej-1 and 2 were classified as moderately resistant (Competitive Grant research, Dr. Ir. Moh. Setyo Poerwoko, MS), and a relatively tolerant Burangrang. Soybean seed planted crops on plots measuring 6 x 4 m, a distance of 0.75 m and a swath spacing of 10 x 40 cm with 2-3 seeds per planting hole, with three replications. As a control each planted soybean genotypes tested in the home screen to avoid infection pathogens to obtain healthy plants, grown in the screen house the Faculty of Agriculture, University of Jember. The severity of soybean leaf rust infection in each genotype was observed at the age of 50 and 70 days after planting (dap) to determine the intensity of the disease using the formula Mc. Kinney (1923 In Tjahjani, 1999) is an $IP = [\sum (nxv) / ZxN] \times 100\%$. Measurement of resistance of soybean genotypes to soybean leaf rust is determined based on international working group of soybean rust (IWGSR). In the measurement of disease severity were observed based notation rating with three digits (Quebral and Opina, 1978), namely (1) digit 1 indicates the position of the leaves on the plant with the notation 1-3 rating (1 = position of the leaves on the lower third of the plant measured ground level, 2 = position of the leaves on the middle third of the plant was measured from ground level and 3 = position of the leaves on the top third of the plant was measured from ground level), (2) digits 2 shows the density of rust lesions on leaves with notation rating 1-4 (1 = no infection is 0 lesions / cm², 2 = density of the rare lesions 1-8 lesions / cm², 3 = density of the lesions was 9-16 lesions / cm², 4 = density of the solid lesion that is more than 16 lesions / cm²), and (3) digits 3 shows the reaction of plants with notation rating 1-3 (1 = no pustules, 2 = no sporulating pustules, 3 = sporulating pustules). Further resistance is based on a scale of 1-5 grouped IWGSR that didiskripsikan in Table 1. Table 1. Description of the grouping of soybean genotypes resistance to leaf rustrating IWGSR soy based code (Abadi, 2000). Skala Description Code Rating IWGSR 1 Imun 111 2 Resistance 122, 123, 132, 133, 222, 223 3 Moderatly resistane 142, 143, 232, 233, 242, 243, 322, 323 4 Moderatly susceptible 332, 332 5 Susceptible 342 122 rating code example shows that the leaves on the lower third of the density of plants infected with rust lesions are rare and no sporulating rust pustules. Biochemical mechanism of resistance of soybean genotypes tested by analyzing the content of phenol compounds and flavonoids which act as phytoalexin compounds. Tests carried out in the laboratory of Molecular Biology University of Jember. Plant leaf sample used in the analysis was obtained from leaf rust-infected plants in each genotype at the age of 50 and 70 dap. Analysis of Phenol and Flavonoid compounds content of each method Kalkonen et al. (1999) and Zhuang et al. (1992). The data were analyzed by ANOVA randomized block design. Further trials carried out by Duncan's multiple range test 5% level. RESULTS AND DISCUSSION Response of soybean genotypes were tested against soybean leaf rust pathogen infection is different. Based on the grouping of plant resistance rating IWGSR code according to the severity of infection by measuring the time the plant was 70 dap in the leaves of the upper third of the plant, indicating that the genotype Unej Unej 1 and 2 were previously reported to be somewhat resistant proved to be quite resistant. Genotypes were previously classified Burangrang apparently tolerant and moderately resistant genotypes including Lokon were previously classified as vulnerable apparently reacted moderately susceptible (Table 2). Tabel 2. Classification code based on the resistance of soybean genotypes rating IWGSR Genotype Code Rating Disease Intensity IWGSR Security Description (persen) Unej-1 322 Moderatly Resistance 46,67 b Unej-2 322 Moderatly Resistance 48,89 b Burangrang 322 Moderatly Resistance 40,00 b Lokon 332 Moderatly Resistance 60,00 a Figures followed by the same small letters in the same column indicates not significant at Duncan's test level of 5%; 322 = The leaves at the top third of the density of plants infected with rust lesions are rare and no sporulating pustules; 332 = The leaves at the top third of the plants infected with rust

lesion density was and there was a little rust sporulating pustules, IWGSR = International Working Group on Soybean Rust. Judging from the intensity of the disease seem that Lokon genotype showed the highest level of severity of the disease intensity of 60.00 percent. Based on the intensity of the susceptible genotype Lokon is actually classified as a disease intensity over 50 percent (0-25 percent = resistant, moderate = 25-50 percent and more than 50 percent = susceptible). According Semangun (2001) in plants are susceptible to a compatible relationship between host and pathogen, so the pathogen can spread in the body of the host without a hitch. The development of the intensity of the disease four soybean genotypes based on the age of the plant was measured in the leaves of the upper third of the plant from the soil surface, shown in Figure 1.

8 7 0 Disease Intensity (%) 6 0 0 5 4 0 Unej 3 0 U1nej 2 0 B2urangrang 0 1 Lokon 0 0 3 4 6 7 5 0 0 Plant Day0s (dap) 0 0 Figure 1. Development image intensity soybean leaf rust disease. Increased soybean leaf rust pathogen infection occurs through the increase in the number of leaves and spread urediospora followed by the development of patches to the leaves on the top line of plant age. Hardaningsih et al. (1986) reported that severe rust pathogen attack occurs when soybean age 50-71 HST. According Sastrahidayat (1989) incubation of soybean leaf rust pathogen is relatively faster on young leaves than old leaves. The response of different soybean genotypes to soybean leaf rust pathogen infection because each genotype has a mechanism to fight infection with the pathogen resistance of different degrees. According Djaparuddin (2000) general mechanisms of plant resistance to pathogens can be divided into mechanical resistance, biochemical and functional. But according Semangun (2001) The resistance between the three biochemical resistance plays a more important than the mechanical resistance and functional. In this study, four soybean genotypes were tested turned out to show the difference in content of phenol compounds and flavonoids. Three genotypes were classified as moderately resistant (Unej 1, 2 and Burangrang Unej) showed an increase in the number and speed of formation of phenol and flavonoid compounds is higher than that somewhat susceptible genotypes or susceptible (Lokon) both in healthy and infected plants (Figure 2 and 3). Based on the results and statistical analysis at the age of 70 HST-infected plants, among the three genotypes showed moderately resistant Burangrang phenol and flavonoid content is higher and the lower Unej 1. Healty Plant Fenol (mg GAE/g) Infected Plants 1 1 8,9 9,7 10, 8 7,4 8,6 5,1 5,3 6,7 7,0 4,7 6,1 4 6 4,3 5,5 5,9 4,2 4,4 0 2 50 dap 70 dap 50 dap 70 dap 50 dap 70 dap Unej Unej Burangrang Lokon Soybean Genotype Figure 2

Content of phenol compounds soybean genotypes 50 and 70 dap Healty Plant Flavonoid (mgQE/g) 0,8 Infected Plant 0,76 0,7 0,6 0,5 0,39 0,47 0,4 0,31 0,32 0,39 0,3 0,21 0,23 0,16 0,21 0,27 0,21 0,25 0,2 0,12 0,1 0,13 0,1 0 50 dap 70 dap 50 dap 70 dap 50 dap 70 dap 50 dap 70 dap Unej 1 Unej 2 Burangrang Lokon Soybean Genotype Figure 3

Content of Flavonoids are compounds of soybean genotypes ages 50 and 70 dap Compared with healthy plants, in each genotype appears also an increase in the number and speed of formation of phenols and flavonoids in the leaves of plants infected with soybean rust. According to Agrios (1986) pathogen infection does stimulate the addition content of chemical compounds (secondary metabolites) in plants that serve as plant defense against pathogen attack. Eternal (2003) also suggested that the pathogen-infected plants generally increased respiration rate. Increased respiration in diseased plants was also followed by increased activity of the pentose series which is the main source Fenol.yang compounds play an important role in plant defense mechanisms against pathogens. Lokon is somewhat susceptible genotypes indicates the amount of phenol compounds and flavonoids lowest respectively 5.90 mgGAE / g and 0.25 mgQE / g, while the highest Burangrang respectively 10.50 mgGAE / g and 0.76 mgQE / g . The rate of formation of these compounds also appear on the moderately resistant genotypes compared to susceptible visible rather than the increase of the content of phenol and flavonoid compounds from the plant ages 50 to 70 dap. According Semangun (1996) on resistant and susceptible plants actually produce phytoalexin equally, but the resistant plant phytoalexin formed faster with more quantity. Eternal (2003) suggested that the phenol compounds which are toxic to pathogens is produced and accumulates more quickly after the infection occurs in resistant varieties compared to susceptible varieties. According to Agrios (1986) increased the concentration of phenol in leaf tissue thought to be responsible for the occurrence of network robustness against pathogens. In pathogen-infected plants generally increased respiration rate. Increased respiration in diseased plants was also followed by increased activity of the pentose series is a major source of phenolic compounds. Due to soybean leaf rust infection decreased production of components (number of pods, number of seeds and seed weight) per plant in all genotypes tested. The decline was mainly on the weight of seeds per plant. Based on the results of statistical analysis Lokon somewhat susceptible genotypes showed the highest seed weight is 6.34 percent (Table 3). The

decrease in production may occur due to soybean leaf rust pathogen infection resulted in many rust spots that occupy the upper and lower surface of the leaf so that the leaf photosynthetic process was not optimal and consequently decreased photosynthate results. According Suprpto (1992) Soybean leaf rust disease can interfere with the process of photosynthesis, so many pods are not fully charged. According to Henn (2004) leaf rust pathogen infection can result in reduced formation of pods, number of seeds, and seed weight.

CONCLUSIONS AND RECOMMENDATIONS Based on the increase in the number and speed of formation of phenol and flavonoid compounds in plants infected with leaf rust pathogen of soybean, soybean genotype resistance Unej 1, 2 and Burangrang Unej against soybean leaf rust infection proved to be controlled by biochemical resistance mechanisms. Therefore the content of phenol compounds and flavonoids can be used as an indicator to detect the resistance properties of soybean genotypes to soybean leaf rust. Unej Genotype-1 and-2 are categorized Unej moderately resistant decreased seed number and seed weight per plant is lower than Lokon rather vulnerable. While the genotype Burangrang difference was not too visible.

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