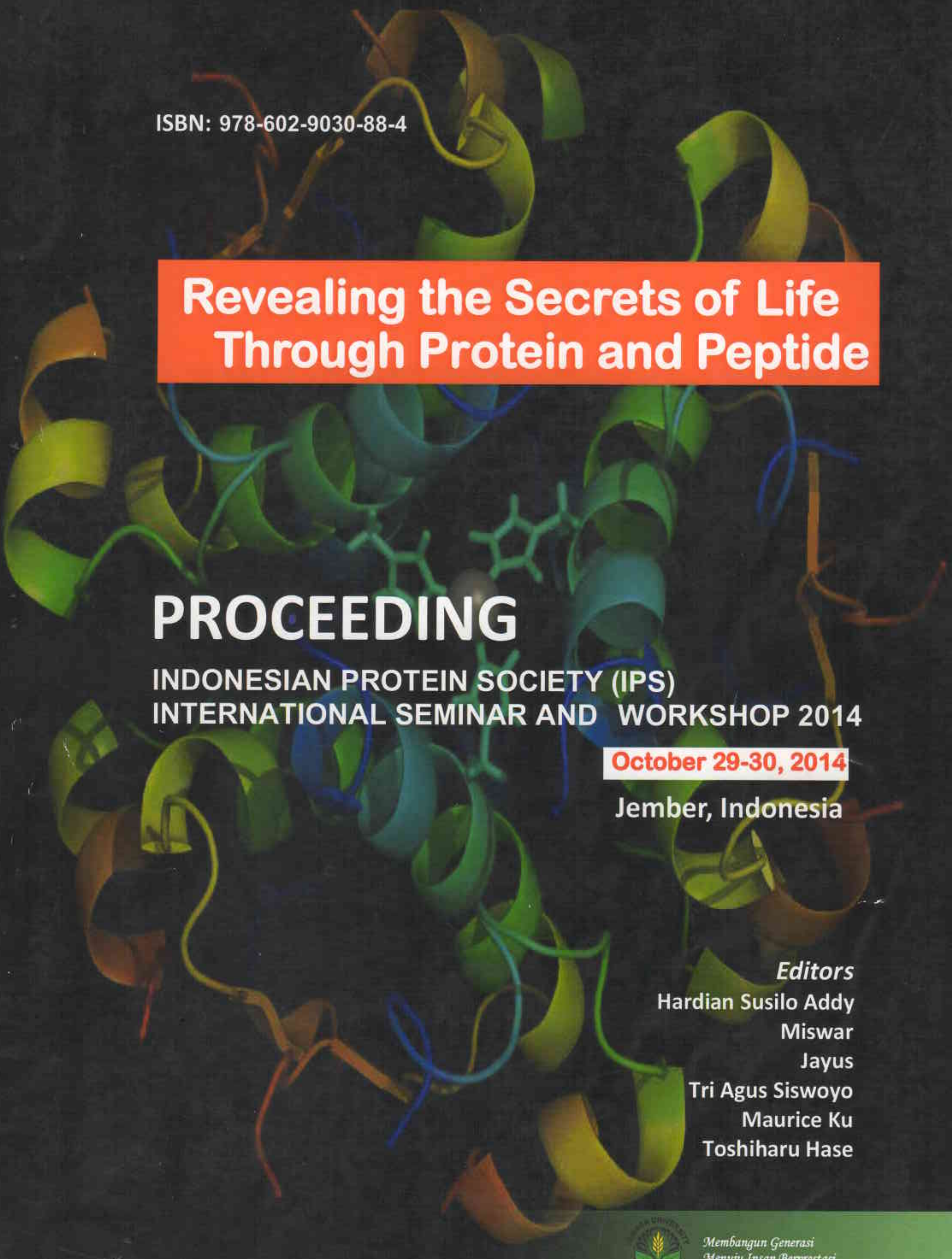
ISBN: 978-602-9030-88-4



Revealing the Secrets of Life Through Protein and Peptide

PROCEEDING

INDONESIAN PROTEIN SOCIETY (IPS) INTERNATIONAL SEMINAR AND WORKSHOP 2014

October 29-30, 2014

Jember, Indonesia

Editors
Hardian Susilo Addy
Miswar
Jayus
Tri Agus Siswoyo
Maurice Ku
Toshiharu Hase

Membangun Generasi Menuju Insan Berprestasi

Center for Development of Advanced Sciences and Technology, University of Jember

Indonesia Protein Society (IPS)

Indonesian Library Catalogue

PROCEEDING
INDONESIAN PROTEIN SOCIETY (IPS)
INTERNATIONAL SEMINAR AND WORKSHOP 2014
Revealing the Secrets of Life Through Protein and Peptide

Editor:
Hardian Susilo addy
Miswar
Jayus
Tri Agus Siswoyo
Maurice Ku
Toshiharu Hase
Bambang Sugiharto

ISBN: 978-602-9030-88-4

Published first: December 2015
By:
UPT PENERBITAN
UNIVERSITAS JEMBER
Jl. Kalimantan 37 Jember 68121

Table of Contents

Indonesian Protein Society
Proceeding of International Seminar and Workshop on Protein
Jember, October 29-30, 2014

Research Article

Biochemical Resistance Mechanism of Several Genotype of Soybean to Rust Diseases

Moh. Setyo Poerwoko, Endang Budi Trisusilowati dan Amarullah

Faculty of Agriculture. University of Jember

Corresponding email: moh_setyo_poerwoko@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 greather than Lokon which was moderate susceptible

Keywords: 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 gliseolin 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 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

Biochemicalmechanismsof soybeanresistancetosoybeanleaf rustwere testedin 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 $IP = [\Sigma (nxv) / ZxN] \times 100\%$. 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 / cm 2, 2 = density of the rare lesions 1-8 lesions / cm 2, 3 = density of the lesions was 9-16 lesions / cm 2, 4 = density of the solid lesion that is more than 16 lesions / cm 2), 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.

Biochemicalmechanism ofresistance ofsoybean genotypestestedby analyzing thecontent ofphenolcompoundsandflavonoidswhichact asphytoalexincompounds. Testscarried outinthe laboratoryof Molecular BiologyUniversity of Jember. Plantleafsampleused in the analysiswas obtainedfromleafrust-infected plantsineachgenotypeat the age of50and70dap. Analysis ofPhenolandFlavonoidcompoundscontent ofeachmethodKalkonenetal. (1999) andZhuangetal. (1992). The data wereanalyzed byANOVArandomized block design. Furthertrialscarried outbyDuncan's multiple range test 5% level.

Table 1. Description ofthe grouping . ofsoybean genotypesresistancetoleaf rustratingIWGSRsoybasedcode(Abadi, 2000).

Skala	Diskripsi	Kode 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

122ratingcodeexampleshowsthatthe leaveson thelower third ofthe densityof plantsinfectedwithrustlesionsare rare andnosporulatingrustpustules.

RESULTSANDDISCUSSION

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

Genotipe	Kode Rating IWGSR	Diskripsi Ketahanan	Intensitas Penyakit (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.

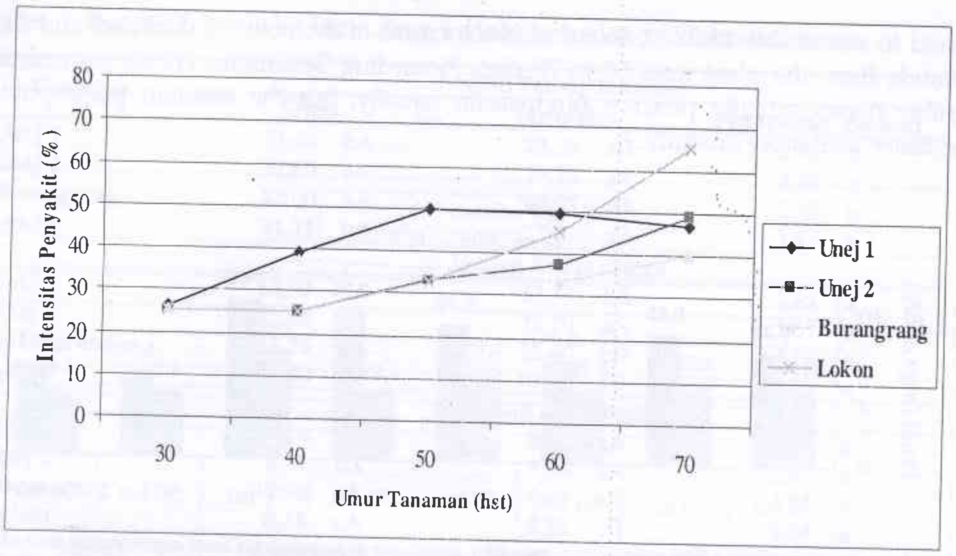


Figure 1. Development image intensity soybean leaf rust disease.

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.

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. 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.

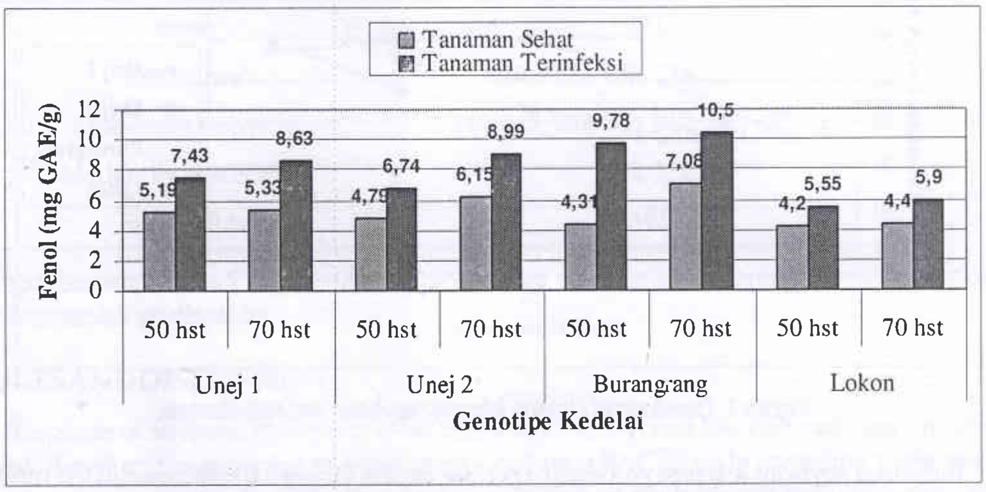


Figure 2. Content of phenol compounds soybean genotypes 50 and 70 dap

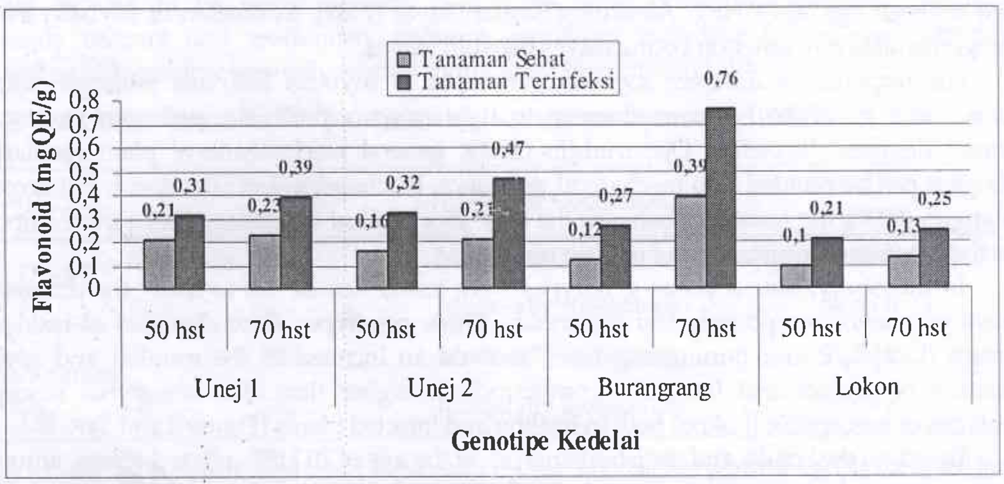


Figure 3. Content of Flavonoids are compounds of soybean genotypes ages 50 and 70 dap

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).

Tabel 3. Pengaruh penyakit karat daun kedelai terhadap produksi kedelai

Genotipe	Sehat		Terinfeksi		Penurunan (persen)	
	Jumlah Polong/tanaman					
Unej 1	25,40	bA	23,33	aB	2,93	a
Unej 2	33,60	aA	29,77	aA	2,94	a
Burangrang	27,00	bA	24,93	aB	2,86	a
Lokon	31,33	aA	26,73	aB	3,88	a
	Jumlah Biji/tanaman					
Unej 1	62,93	bA	57,47	bA	2,82	a
Unej 2	78,62	aA	72,80	aA	2,69	a
Burangrang	63,33	bA	52,40	bB	4,18	a
Lokon	80,80	aA	68,40	aB	3,97	a
	Berat Biji (g)/tanaman					
Unej 1	10,14	bA	8,29	aA	4,22	b
Unej 2	8,47	cA	7,84	aA	2,67	c
Burangrang	12,36	aA	9.09	aB	5,35	a
Lokon	8,38	cA	5,28	bB	6,34	a

Angka yang diikuti dengan huruf kecil yang sama pada kolom yang sama menunjukkan tidak berbeda nyata pada uji Duncan's taraf 5%; Angka yang diikuti dengan huruf besar yang sama pada baris yang sama menunjukkan tidak berbeda nyata pada uji Duncan's taraf 5%.

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 Suprapto (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

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.

REFFERENCES

Abadi. A.L. 2000. Epidemologi dan Strategi Pengelolaan Penyakit Tumbuhan. Lembaga Penerbitan Fakultas Pertanian Universitas Brawijaya. Malang

__________ 2003. Ilmu Penyakit Tumbuhan II. Bayumedia Publishing. Malang

Adisarwanto, T. 2005. Budidaya Kedelai dengan Pemupukan yang Efektif dan Pengoptimalan Peran Bintil Akar. Penebar Swadaya. Jakarta

Agrios, G.N. 1986. Ilmu Penyakit Tumbuhan. Edisi Ketiga. Terjemahan Munzir Busnia. Gaja Mada University Press. Yogyakarta

Djaparuddin. 2000. Dasar-dasar Pengendalian Penyakit Tanaman. Bumi Aksara. Jakarta

Gomez, K.A. dan A.A. Gomez. 1995. Pengantar Statistik untuk Penelitian Pertanian Terjemahan E. Sjamsudin dan J.S. Baharsjah dari Statistical and Prosedural Reseacrh of Agricultural. UI-Press. Jakarta

Hardaningsih, S., P. Nursamsi, dan Sudarmadi. 1986. Hubungan Antara Serangan Jamur karat (*P. Pachyrhizi.* Syd) dengan Hasil Kedelai. Risalah Seminar Hasil penelitian Tanaman Pangan Th. 1993. Balai Penelitian Tanaman Pangan, Malang. 72-75

Henn, A. 2004. Soybean Rust. Extention Plant Physiologist. Missisipi State University

Kahkonen. M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha J.P, K. Pihlaja, dan T.S. Kujala. 1999. Antioxidant Activitiy of Plant Extract Containing Phenolic Compounds. Jurnal of Agriculture and Food Chemistry, 47: 3954-3962.

Oka, I.N. 1993. Pengantar Epidemi Bagi Penyakit Tanaman. Program Nasional Pengendalian Hama Terpadu. BAPPENAS. Gajah Mada University Press. Yogyakarta

Quebral, F.C. and O.S. Opina. 1978.Technique in determinating pest intensitas in legumes. Pp: 495-498, in Susan S.L and Pura J.L (Eds). Research Techniques in Crops. PCARRD Book Series No. 35/1985. PCARRD. Phillippines

Robinson,T. 1995. Kandungan Organik Tumbuhan Tinggi. Terjemahan Kosasih Padmawinata dari The Organic Constituents of Higher Plants (1991). Penerbit Institut Teknologi Bandung. Bandung

Salisbury, F.B dan C.W. Ross. 1995. Fisiologi Tumbuhan. Terjemahan Diah R Lukman dan Sumaryono dari Plant of Physiology. ITB Bandung Sastrahidayat, H.I.R 1989. Kajian biologi dan ekologi *Phakopsora pachyrhizi* Syd pada tanaman kedelai. Universitas Brawijaya, 1(2): 38-46

Sastrahidayat, H.I.R 1989. Kajian biologi dan ekologi *Phakopsora pachyrhizi* Syd pada tanaman kedelai. Universitas Brawijaya, 1(2): 38-46

Semangun, H 1996. Ilmu Penyakit Tumbuhan. UGM Press University. Yogyakarta

______ 2001. Pengantar Ilmu Penyakit Tumbuhan. UGM Press University. Yogyakarta

Sinaga, M.S. 2003. Dasar-dasar Ilmu Penyakit Tumbuhan. Penebar Swadaya. Jakarta

Suprapto, H. 1992. Dasar-dasar Ilmu Penyakit Tumbuhan. Penebar Swadaya. Jakarta

Tjahjani, A. 1999. Pengaruh Penyakit Karat Daun (*Phakopsora pachyrhizi* Syd). terhadap Penurunan Daya hasil 40 Genotipe Kedelai

Zhuang. X.P., Y.Y. Lu, and G.S. Yang. 1992. Extraction and Determination of Flavonoid in Ginkgo. Chinese Herbal Medicine. 23:122-124

Anggota APPTI No. 036/KTA/APPT/2012
Anggota IKAPI No. 127/JTI/2011

ISBN: 978-602-9030-88-4

Jember University Press
Jl. Kalimantan 37 Jember 68121
Telp. 0331-330224, psw. 0319
***E-mail*: upt-penerbitan@unej.ac.id**