The Effect of Fusaric Acid Application on the Lignin and Suberin Formation as Resistance Indicator on Tomato

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Abstract: Fusaric acid (=FA) is a phytotoxin produced by *Fusarium oxysporum*, pathogen of wilt disease on tomato. A concentration of FA causes death of plant. However, low concentration will elicit various plant defense responses and inhibit pathogen growth. The research focused on the observation of lignin and suberin formation after application of FA on tomato in different concentration. This research was caried out at elementary laboratory of Agriculture Faculty of Muhammadiyah University of Parepare, Parepare and Micology Laboratory of Gadjah Mada University, Yogyakarta. Four concentrations of FA (sigma) 0, 25, 50, 75, and 100 ppm were used in this research, whereas inoculation with *Fusarium oxysporum* was served as control. Lignin and suberin formations in plant cell as structural resitantance response were analyzied by using microtechnique protocol. The result of histological analysis showed that treatment of *F. oxysporum*, 10 ppm, 50 ppm, 75 ppm, and 100 ppm of FA, caused a change in lignin content of tomato cell on 10 – 40 days after application and followed also by necrotitation. The application of 25 ppm FA increases the lignin and the suberin content without necrosis on 20 and 30 days after application.

Keywords: Fusaric acid; lignin; suberin; tomato resistance

1. Introduction

Fungal pathogens produce a number of low molecular weight of secondary matabolites which are toxic to plants. Host selective phytotoxin, produced only by a few fungal species, are exclusive toxic to host-plant, whereas the non-host specific phytotoxin also cause symptoms on many plants which naturally cannot be infected by the toxin-produced pathogen. FA (5-butylpicolinic acid) as a non-host spesific phytotoxin, produced by *Fusarium oxysporum,* was one of the first fungal metabolites implicated in the pathogenesis of wilt symptoms of tomato (Bouizgarne *et al*., 2004)

Toxic concentration of fusaric acid will cause the decrease of plant cell viability. The plant will be wilt up to die, whereas at non-toxic concentration could induce plant defence responses. Bouizgarne *et al.* (2006) said that fusaric acid at non-toxic concentration was able to induced the synthesis of phytoalexin, a classic delayed plant response to pathogen. FA could also induced rapid responses putatively which involved in signal transduction, such as production of reactive oxygen species, and increasing in cytosolic calsium and ion chanel current modulation.

F. oxysporum was found to induced classic events described in the initial phase for recognition of the plant-phatogen interaction, which is crucial for recognition of the fusaric acid by the plant. The interaction could biochemical process to induced physic and chemical defence, such as lignin and phenolic compounds (Agrios, 2005). Therefore, the objective of the study was to designed to effect of FA concentration to the change of lignin and suberin on tomato.

2. Materials and Methods

2.1 Location and Time

This research was conducted at two places, it were in elementary laboratory of Agriculture Faculty, Muhammadiyah University of Parepare, South Sulawesi and Micology Laboratory Agriculture Faculty Gadjah Mada University, Yogyakarta, from March to September 2014.

2.2 Aplication of Fusaric Acid on Tomato Seed

Local variety of tomato seed were used in this research. FA solution was prepared by dissolving each 0, 10, 25, 50, 75 and 100 mg of FA in 1 l of sterile distille water. Whereas *F. oxysporum* consisted of spore suspension obtained from 7 days old culture on Czapek Dox Medium. 21 days of cultivation of tomato seed were transplanted in the polibags (plastic pot). Individual tomato seeds were pouring in 40 ml FA suspension of 10, 25, 50, 75, 100 ppm concentration and spore suspension (1 x 10^7 spore/ml) for 15 minutes. Sterile distille water as control of treatment (Ambar *et al,* 2013).

2.3 Histological Analysis After Fusaric Acid Application

Histological analysis of tomato plant was revealed on 10, 20, 30, and 40 days after FA application (Hwang, 1995 and Martanto *et al.,* 2003). Extensive formation of lignin and suberin in plant cell were measured by using a raster image program.

All samples (roots and stems) were cut along 1 cm, fixed in FAA solution then cut using a microtome. Slices were soaked first in (phloroglucinol 0.5-1.0 g + 50 ml of

95% ethanol) for 15 minutes, then soaked in a mixed solution of HCl + distilled water (1:3) for 5 minutes. Performed semipermanent slices were made by using 87% glycerin, and then observed microscopically and photographed. Tissue containing lignin has a purple colour (Sass, 1971 and Ruzin, 1999). For detection of suberin, tissue slices were soaked in Sudan IV stain (0.5 g dissolved 100 ml of 80% ethanol) for 5 minutes, immersed in 50% ethanol for 5 minutes, then saturated 87% glycerin. Cell contained suberin will be red colour when observed under a microscope (Sass, 1971 and Ruzin, 1999).

3. Results and Discussion

3.1. Histological Analysis of Tomato Tissue after Fusaric Acid Application

Effect of FA concentration (10 ppm, 50 ppm, 75 ppm, 100 ppm) and *F. oxysporum* were caused by change in lignin and suberin that also followed by necrotitation in plant tissue. Application of 25 ppm FA increased the lignin and suberin formation without necrosis on 20 and 30 days after application.

Effect of FA concentration (10, 25, 50, 75, and 100 ppm) and inoculated with *F. oxyspurum* and controls are presented in Table 1. It is obtained their symptoms are almost evenly on day 10^{th}, 20^{th}, 30^{th}, and 40^{th} after application, i.e. the emerge of lignin, suberin and necrosis were almost side by side in the plant cell.

Application of FA (10, 25, 50, 75, 100 ppm) and inoculated *F. oxysporum* were used in the study of lignin and suberin formation. The Cell of containing lignin and suberin became purple and red colour. Observation of lignin, suberin, and necrosis of plant cell were presented in Figure 1.

Concentrations 10 and 25 ppm of FA induced the formation of lignin without necrosis on 20 and 30 days after application, while the suberin formation is often accompanied by the appearance of necrosis, especially in the age of 10 and 30 days after application. However, there are similarities in the process of lignin formation. Optimal concentration of FA for suberin was 25 ppm, that observed on 20 – 30 days after application, with an area of (93,70 x 47,30 μm dan 85,40 x 82,00 μm) respectively.

3.2. Discussion

The observations in plant cell, either treated with fusaric acid and inoculated with *F.oxysporum*, it appeared that the lignin and necrosis formed almost in all treatments. Lignin was generally formed in the cortex, epidermis, and xylem tissue. Lignin was often formed on the transport tissue than in the epidermal cells. This happened was because the infection of fusaric acid and *F. oxysporum* starting from the plant roots, so that the first root tissue responded by forming lignin or necrosis. Sticher *et al*. (1997) states that local infections can increase the resistance to pathogen infection. The resistance expressed at the beginning of the inoculation place and was usually preceded by a local necrosis in infected tissue.

Lignin is formed as a response to penetration by pathogens or caused by mechanical damage (Vance *et al*., 1980 and Sticher *et al*., 1997). Lignin formation was occur as a result of treatment with FA in concentration of 10-100 ppm and *F. oxysporum*.

Table 1. The effect of fusaric acid concentration (ppm) of the establishment size lignin, suberin and necrosis (µm) in tomato cells on day 10-40 after application.

Time (day)	Treatment	Histological Analysis (µm)			
		Lignin	Necrosis	Suberin	Necrosis
10	*F.o*	40.86 x 39.21	25.76 x 29.07	34,08 x 11,12	81,40 x 26,49
	10 ppm	56.27 x 45.67	14,50 x 21,80	47,40 x 6,43	34,10 x 5,08
	25 ppm	45,60 x 128,30	20,76 x 12,98	68,90 x 41,60	102,80 x 15,52
	50 ppm	78,60 x 39,81	28,76 x 42,01	29,50 x 46,90	-
	75 ppm	87,60 x 30,25	-	54,80 x 13,20	85,90 x 4,71
	100 ppm	69,80 x 28,86	39,50 x 12,10	44,70 x 8,96	-
	SW	-	-	-	-
20	*F.o*	98,02 x 16,86	49,89 x 25,78	22,30 x 2,49	32,45 x 8,61
	10 ppm	248,40 x 130,28	-	89,30 x 43,94	20,00 x 4,30
	25 ppm	306,10 x 128,30	-	93,70 x 47,30	42,60 x 10,19
	50 ppm	145,70 x 36,03	30,32 x 11,53	-	47,60 x 8,17
	75 ppm	72,30 x 35,08	42,20 x 24,68	-	64,50 x 14,90
	100 ppm	82,80 x 30,53	60,10 x 31,20	79,60 x 51,24	166,10 x 15,56
	SW	-	-	-	-
30	*F.o*	72,29 x 42,02		51,20 x 47,70	124,90 x 6,13
	10 ppm	188,00 x 69,28	-	-	47,70 x 5,50
	25 ppm	204,90 x 80,32	-	85,40 x 82,00	81,34 x 26,04
	50 ppm	133,90 x 51,57	44,40 x 23,85	-	26,50 x 14,93
	75 ppm	92,00 x 40,37	22,30 x 22,24	71,20 x 18,00	138,10 x 11,92
	100 ppm	75,60 x 42,48	27,60 x 53,20	-	170,90 x 7,10
	SW	42,80 x 48,23	-	-	11,98 x 29,78
40	*F.o*	-	-	-	-
	10 ppm	119,20 x 53,27	35,97 x 52,80	46,20 x 25,66	111,60 x 24,84
	25 ppm	122,70 x 81,54	16,70 x 38,50	122,60 x 8,83	-
	50 ppm	177,00 x 66,06	78,00 x 60,35	102,50 x 42,30	33,67 x 23,02
	75 ppm	166,50 x 81,00	155,10 x 82,00	26,10 x 23,90	48,80 x 18,40
	100 ppm	160,70 x 48,56	116,40 x 10,14	138,90 x 77,40	34,20 x 29,78
	SW	89,94 x 36,56	47,38 x 36,79	24,76 x 15,79	46,23 x 19,78

Remarks: SW = sterile water

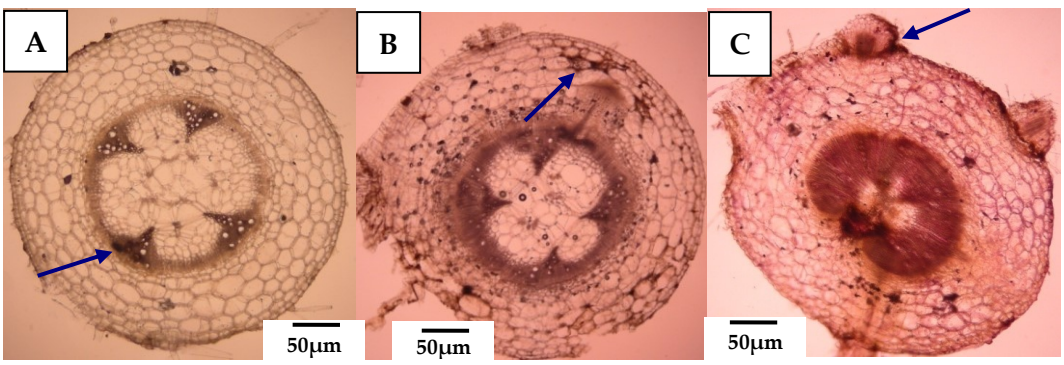

Figure 1. Histology tomatoes containing lignin (A), necrosis (B) and suberin (C) after treated with fusaric acid.

Bouizgarne *et al*. (2006) states that application of FA at the concentration of 10^{-7}M cause an increase of H_2O_2. Activity of H_2O_2 associated with the formation of lignin peroxidase. The coniferyl alcohol changed into lignin through the process of oxidation of phenolic hydroxyl groups. The process is the task of peroxidase, causing fenoxiradical into monomers and dimers, then dehidrogenated and eventually become lignin.

The incidence of necrosis in plant tissue caused by uncapability of plant cells defense themselves from infection pathogens or chemical compounds. Necrosis occurs due to damage of cells caused by the reaction of chemical compounds released by pathogens. According to Suganda (2000 *cit*. Soesanto & Rahayuniati, 2009), the resistance reaction can emerge from the results of their induced resistance expression, which is the result of a series of gene expression resistance activated by external stimulation.

In the 10 and 25 ppm of FA concentration, the compound was probably to be absorbed by the tomato seedlings and be a signal to the tomatoes tissue, so that the tomatoes tissue responded by creating lignin or suberin. In higher FA concentration, the signal was sent slower in response to form lignin and suberin. In this interaction, necrosis formation taken earlier. The lignin and suberin formation are often coexist with necrosis.

FA of 25 ppm is the ideal concentration to activated H_2O_2. The H_2O_2 is a peroxidase donor for the formation of lignin. According to Bouizgarne *et al*. (2006) and Kuzniak *et al*. (1999). The application of FA at non-toxic concentrations cause increased/activated O_2 and H_2O_2. The peroxidase serves as a donor of H_2O_2 and as server in lignification (Lewis & Yamamoto *cit*. Polle *et al*., 1994). Peroxidase acts as the last enzyme in the formation of lignin.

Inoculation of tomato inoculated with *F. oxysporum* gave a responses of lignin formation which is always accompanied by the appearance of necrosis. This became the basis of distinguishing with FA treatment.

Pattern formation of suberin in tomato cell effected by FA concentration application. However, the application in FA concentration of 25 ppm is still showing the best results by setting up an area 85,40 x 82,00 µm of suberin, looked at 20 days after application. This indicated that the concentration of 25 ppm is the ideal concentration to induced the lignin and suberin formation.

4. Conclusion

The effect of FA concentration (10, 25, 50, 75, 100 ppm) and *F. oxysporum* cause lignin, suberin and necrosis were formed in tomato plant, although application of FA on Tomato provide better structural responses to induced lignin and suberin formation than application *F. oxysporum*. The FA in concentration of 25 ppm give best result in lignin and suberin formation without necrosis, especially on 20 and 30 days after application.

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