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In Vivo Study: Characterization of Mutants *Vanilla planifolia* Andrews Resistant To Fusarium Wilt Disease Based On Analysis of the Lignin and the Phenol Content

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Abstract: *The most production constrain on Vanilla planifolia plantation recently has been caused by foot rot disease that later influence in decreasing the yield product. This disease is caused by Fusarium oxysporum f. sp. vanillae (Fov). So far, the disease has not been successfully prohibited although some experiments had been conducted. The use of foot rot resistant cultivar has been introduced, which has high yield expected as one alternative method for controlling this disease. A resistant vanilla plantlet to Fov has been initiated by in vitro selection on medium containing fusaric acid (FA) on different concentration (90, 100, 110, and 120 ppm). In vivo, inoculation of Fov fungal isolates in vanilla mutants has been performed previously. The long-term goal in the overall study was to obtain Fov-resistant mutant seedlings. Specific targets to be achieved were characterization of V. planifolia mutant by in vivo. The stages of this research include: resistance analysis, lignin thickness and the phenol content of mutant resistant to Fusarium wilt disease by in vivo. The results showed that in vivo condition using concentration of FA of 110 ppm was effective for suppressing the growing of Fov, by intensity up to 25%, compared to the concentration of 90 ppm and 100 ppm respectively. In other words by using 110 ppm fusaric acid could increased the category criteria to resistant. There was a significant increased in the total of lignin thickness, and the phenol content, overall in line with the rising FA concentration.*

Keywords: *Vanilla planifolia* Mutant; lignin thickness; the phenol content; *Fusarium oxysporum* f.sp. *vanillae, in vivo*

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I. Introduction

Vanilla is an epiphytic plant and belongs to the familia Orchidaceae. The genus of vanilla consists of about 150 species, but the only economically valuable are only 3 species, namely *V. planifolia* Andrews, *V. tahitensis* J. Wi Moore and *V. pompona* Schieda (Besse *et al*., 2004; Minoo *et al*., 2008; Umamaheswari & Mohanan, 2011). The most widely cultivated species, especially in Indonesia are *V. planifolia* Andrews (Anandaraj *et al*., 2005; Rajeev P & Dinesh R, 2005; Palama *et al*., 2010).

One of the plantation commodities in Indonesia and in Lampung Province in particular, with a fairly high economic value and has a good name in the international market is vanilla. United Nations Development Program (UNDP), recommends that Indonesian vanilla is no different from "Bourbon vanilla" which has an excellent commodity image in the international community (Umamaheswari & Mohanan, 2011). Vanilla is widely used in the food, beverage and medicine industries; at this time vanilla is being developed its use as a raw material for making perfume in the form of tincture or absolute (Kalimuthu *et al*., 2006; Abebe *et al*., 2009; Mengesha, 2012).

The foot rot disease on vanilla is the most crucial disease caused by *Fusarium oxysporum* f. sp. *vanillae* (*Fov*), which is the most limitation and up to know days is not well managed yet. One an alternative way to control foot rot disease could be done by using a cultivar, which is resistant to this disease (Nurcahyani, 2014). Fusaric acid (FA) is a metabolite produced by several fungal species of the Fusarium genus. Chemically the FA is called 5-n-butylpicolinic acid. The use of FA as a selecting agent in in vitro selection may produce an insensitive mutant network of FA, so that once regenerated into the plant may produce strains resistant to pathogen infection (Arai dan Takeuchi, 1993). Identification of mutants or variants that are insensitive to AF with in vitro selection was performed on tomato plants (Toyoda *et al.*, 1984), pisang (Morpurgo *et al*., 1994; Matsumoto *et al*., 1995), gladiol (Remotti *et al*., 1997), nanas (Borras *et al*., 2001), dan vanili (Nurcahyani *et al*., 2012; Nurcahyani *et al*., 2014; Nurcahyani, 2017), *Spathoglottis plicata* (Nurcahyani, 2016a, Nurcahyani,

2016b). The results of these researchers showed that somaclonal from the regeneration of cell mass that is resistant to the toxin is also resistant to pathogens.

Based on previous research outcomes obtained in the form of *V. planifolia* resistant mutant candidates *Fov*. Mutant of *V. planifolia* subsequently acclimatized in greenhouse, so that obtained mutants that have adapted to the environment outside (*in vivo*). The resistance analysis of *V. planifolia* and mutant macropropagation was subsequently carried out to obtain the resistance of the *Fov* resistant *V. planifolia in vivo*. Characterization of mutants in the form of lignin thickness and phenol content is carried out further to obtain the specific character of *Fov* resistant mutant.

II. Material and Methods

Materials research in the form of *Vanilla planifolia* Andrews obtained from previous research. For resistance analysis, lignin thickness and phenol content used 70% alcohol, Potato Dextrose Agar (PDA), gallic acid, Folin-Ciocalteau reagent, Na_2CO_3, sodium carbonate, 80% ethanol, phloroglucinol, FAA, 1% safranin, balsam canada.

For the total phenol analysis using Singleton & Rossi (Aberouman & Deokule, 2008) method. For the preparation of standard calibration curve of phenol compound, gallic acid was used as standard solution, as control used aquades. After the absorption value is known then made standard curve, and seen the regression equation between the concentration of gallic acid and the value of absorption. The mutant vanilla extract is prepared according to Ozygi *et al.* (2007). Vanilla weighing 2 g was crushed using a glass mortar and dissolved in 25 mL of ethanol 80% (*Sigma Chemical* Co.). The solution was then centrifuged at 13,000 rpm for 15 minutes, then taken supernatant. After that, the supernatant was taken as much as 0.5 mL into a 100 mL flask, then added 250 µL of Folin-Ciocalteu reagent, after sterilizing for 5 min and then adding 1 mL of Na_2CO_3. After mixing, it was put into a cuvette with a volume of 5 mL and observed an absorption value at a wavelength of 765 nm using a spectrophotometer (Beckman DU-65), as a control used by aquades. Based on the value of uptake and then determined the content of phenol compounds based on the equation of gallic acid regression is the relationship between absorption value of vanilla extract and series of concentration of gallic acid.

Observation of lignification on cross-section of vanilla rod by means of safranin painting (Ruzin, 1999). Vanilla is removed then the stem is cleaned. The cleaned rods are first fixed by soaking in the FAA and stored for 24 hours. The stem is further clipped in the center of the cork, and sliced with a microtom sliding transversely with a thickness of 5-10 µm. The cross sections were soaked in aqueous safranin (1% w / v) for 1.5 hours, then rinsed with distilled water. The rinsed stems were soaked in a 25% ethanol solution for 2-5 minutes then immersed in safranine and dried. After drying, the stem cut is placed on top of the preparatory glass and covered with a glass cover. Next glass preparations were observed under a microscope with magnification 400 times. The marked trunk network will appear pink.

Qualitative information as the result on this research was consisted as narratives descriptive and supported by photographs. After that, data's were statistically analyzed by Completely Randomized Design. As quantitatively data's from each parameter measured, were compiled and statistically analyzed by analysis of variance (ANOVAs). If the result showed a significantly different, then was continued analyzed by using Duncan Multiple Range Test (DMRT) analysis with accuracy 95%.

III. Results and Discussion

Prior to observation of the analysis of total phenol compounds in vanilla for each treatment, a standard curve measurement was firstly done using gallic acid. This curve measurement was performed to estimate the total phenol quantity by linear regression (Aberouman & Deokule, 2008). From this standard curve measurement, linear regression equation (y = 0,003x-0,006) has high positive correlation (R_2 = 0.997). This shows that the diversity is homogeneous between the concentration of gallic acid and absorbance. Based on the standard curve of the gallic acid then can be found the total phenol content of each treatment based on the equation of the regression line (Table 1).

Table 1. Total phenol content (%) of control and fusaric acid (90, 100, and 110 ppm)

Treatment (ppm)	Average total phenol content (%)
0 ppm	$5,50 \pm 0,03^a$
90 ppm	$6,45 \pm 0,02^b$
100 ppm	$6,63 \pm 0,03^c$
110 ppm	$6,98 \pm 0,02^d$

Description: The numbers followed by unequal letters on one column are significantly different based on the Duncan test at 95% confidence degree, after being transformed to √x +

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Based on Table 1, it is clear that the increase in total phenol content from about 5.50% in control, increased to 6.45% at FA 90 ppm, followed by 6.63% at 100 ppm and 6.98% at 110 ppm. This proves that due to the higher concentration of FA stress, the more the total phenol content is produced.

The results of this study are similar to the results of research conducted by Khan *et al*. (2005) in Chickpea plants infected with *Fusarium oxysporum* f.sp. *ciceris*. From the research it is known that there is a total increase of phenol about 16-17%. Total phenol levels were also reported by Harni *et al*. (2012), on patchouli plants infected by endophytic bacteria and nematodes indicate that there is an indication of increased phenol content by *Achromobacter xylosoxidans*. According to Bouizgarne *et al*. (2006), FA at non-toxic concentrations (10^{-7}) stimulates the formation of H_2O_2 which is strongly associated with peroxidase enzymes. Furthermore, this enzyme will oxidize phenol compounds.

Another parameter that can indicate the resilience of vanilla is the occurrence of lignification in the carrier file network. To detect the occurrence of lignification, cross sectional observation of vanilla rods using phloroglucinol reagent. According to Bouizgarne *et al.* (2006), the formation of lignin involves the role of peroxidase enzymes. Addition of FA at a non-toxic concentration (10^{-7}), causing an increase in peroxide (H_2O_2). Peroxide is a peroxidase enzyme donor for the formation of lignin. The increased peroxide will also increase the activity of peroxidase enzymes that play a role in the formation of lignin.

The results showed that both the controlled vanilla and vanilla rods were treated with lignification. Thus, the effect of FA treatment can be detected by effect through measurement of lignin thickness on xylem wall (Table 2).

Table 2. shows an increase in lignin thickness in xylem cell wall at a treatment of 90 ppm 25,60 μm; 100 ppm 29,27 μm; and 110 ppm 20,40 μm compared to 11,39 μm control. The higher the FA concentration the lignin gets thicker. This suggests that the vanilla responds to resistance by adding thick lignin after being treated with the FA. The plant resistance system depends on the interaction of host, pathogen, and environment. Lignin is a plant-resistant system that functions to inhibit pathogens and is formed by response to penetration by pathogens or mechanical damage (Sticher *et al*., 1997).

Table 2. Mean of lignin thickness (μm) of control vanilla and fusaric acid treatment (90, 100, and 110 ppm)

Treatment Mean lignin thickness (μm), N: 30	Treatment Mean lignin thickness (μm), N: 30
0 ppm	$11,39 \pm 0,04^{a}$
90 ppm	$25,60 \pm 0,08^{b}$
100 ppm	$29,27 \pm 0,05^{c}$
110 ppm	$20,40 \pm 0,06^{d}$

Description:The numbers followed by unequal letters on one column are significantly different based on the Duncan test at 95% confidence degree, after being transformed to √x + 1

IV. Conclusion

Based on the results and discussions that have been described in advance can be concluded as follows. The increased concentration of fusaric acid increases the total phenol content, peroxidase activity, lignin thickness, and total chlorophyll content, chlorophyll a, and chlorophyll b in resistant vanilla planlet *Fusarium oxysporum* f.sp. *vanillae*.

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References

[1]. Abebe Z, Mengesha A, Teressa A, & Tefera W. 2009. Efficient *In Vitro* Multiplication Protocol for *Vanilla planifolia* Using Nodal Explants in Ethiopia. *African Journal of Biotechnology* 8 (24): 6817-6821.

[2]. Aberouman AA & Deokule SS. 2008. Comparison of Phenolic Compounds of Some Edible Plants of Iran and India. *Pakistan Journal of Nutrition* 7: 582-585.

[3]. Anandaraj M, Rema J, Sasikumar B, & Suseela-Bhai R. 2005. *Vanilla (Extension Pamphlet).* Printers Castle, Kochi. 11p.

[4]. Besse P, Da Silva D, Bory S, Grisoni M, Le Bellec F, & Duval MF. 2004. RAPD Genetic Diversity in Cultivated Vanilla: *Vanilla planifolia*, and Relationships with *V. tahitensis* and *V. pompona*. *Plant Science* 167: 379-385.

[5]. Boras O, Santos R, Matos A, Cabral P, & Arzola RS. 2001. A First Attempt to Use A Fusarium subglutinans Culture Filtrate For The Selection of Pineapple Cultivar to Fusariose Disease. *Plant Breeding* 120(5): 345-438.

[6]. Bouizgarne, B, Bouteau HEM, Frankart C, Reboutier D, Madiona K, Pennarun AM, Monestiez M, Trouverie J, Amiar Z, Briand J, Brault M, Rona JP, Ouhdouch Y, & Hadrami EI. 2006. Early Physiological Responses of *Arabidopsis thaliana* Cells to Fusaric Acid : Toxic and Signallling Effects. *New Phytologist* 169: 209 – 218.

[7]. Harni R, Supramana, Sinaga MS, Giyanto, & Supriadi. 2012. Mekanisme Bakteri Endofit mengendalikan nematoda *Pratylenchus brachyurus* Pada Tanaman Nilam. *Bul. Littro* 23 (1): 102-114.
[8]. Kalimuthu K, Senthilkumar R & Murugalatha N. 2006. Regeneration and Mass Multiplication of *Vanilla planifolia* Andr.- A Tropical Orchid. *Current Science. Vol. 91. No.10*: 1401-1403.
[9]. Khan IA, Alam SS, Haq A, & Jabbar A. 2005. Biochemistry of Resistance in *Chicpea* Against Wilt Disease Caused by *Fusarium oxysporum* f. sp. *ciceris*. *Pak. J. Bot*. 37: 97-104.
[10]. Matsumoto K, Barbosa ML, Souza LAC, & Teixeira JB. 1995. Race 1 Fusarium Wilt tolerance on banana plants selected by Fusaric Acid. *Euphytica* 84: 67-71.
[11]. Mengesha A, Ayenew B, Gebremariam E, & Tadesse T. 2012. Micro-Propagation of *Vanilla planifolia* Using Enset (*Ensete ventricosum* (Welw, cheesman)) Starch as a Gelling Agent. *Current Research Journal of Biological Sciences* 4(4): 519-525.
[12]. Minoo D, Jayakumar VN, Veena SS, Vimala J, Basha A, Saji KV, Babu KN, & Peter KV. 2008. Genetic Variations and Interrelationships in *Vanilla planifolia* and Few Related Species as Expressed by RAPD Polymorphism. *Genet. Resour Crop Evol* 55: 459-470.
[13]. Morpurgo R, Lopato SV, Afza R, & Novak FJ. 1994. Selection parameters for Resistance to *Fusarium oxysporum* f.sp. *cubense* race 1 and 4 on Diploid Banana (*Musa acuminata* Colla). *Euphytica* 75: 121-129.
[14]. Nurcahyani, E., Hadisutrisno, B., Sumardi, I., & Suharyanto, E. 2014. Identifikasi galur planlet vanili *(Vanilla planifolia Andrews)* Resisten terhadap infeksi *Fusarium oxysporum* f. sp. *vanillae* hasil seleksi *in vitro* dengan asam fusarat. *Prosiding Seminar Nasional: "Pengendalian Penyakit Pada Tanaman Pertanian Ramah Lingkungan".* Perhimpunan Fitopatologi Indonesia Komda Joglosemar-Fakultas Pertanian UGM. ISBN 978- 602-71784-0-3./2014 Hal. 272- 279.
[15]. Nurcahyani E, Sumardi I, Hadisutrisno B, & Suharyanto E. 2012. Supression of Development of Vanilla Foot Rot Disease (*Fusarium oxysporum* f. sp. *vanillae*) Throught In Vitro Fusaric Acid Selection. *Journal of Tropical Plant Pests and Diseases.* Acreditation of DIKTI No.110/DIKTI/Kep/2009. ISSN: 1411-7525. Vol. 12 /No. 1: 12-22.
[16]. Nurcahyani E, Sumardi I, Hadisutrisno B and Suharyanto E. 2017. DNA Pattern Analysis of *Vanilla planifolia* Andrews Plantlet Which Resistant To *Fussarium oxysporum* f. sp. *vanillae. WJPLS* (*3*) *4: 27-34.*
[17]. Nurcahyani E, Rochmah Agustrina, Erdi Suroso, Gardis Andari. 2016b. Analysis of Peroxidase Activity and Total Phenol from *Spathoglottis plicata* Bl Plantlet Toward to *Fusarium oxysporum. International Journal of Applied Agricultural Sciences* 2(6): 79-82.
[18]. Nurcahyani E, R. Agustrina, T. T. Handayani. 2016a. The Protein Profile of the Plantlets of *Spathoglottis plicata* Bl. Induced Resistance to *Fusarium oxysporum*. *Journal of Plant Sciences* 4(5): 102-105.
[19]. Ozygi II, Kahraman MV & Ercan O. 2007. Relation Between Explant Age, Total Phenol and Regeneration Response in Tissue Cultured Cotton (*Gossypium hirsutum* L.). *African Journal of Biotechnology 6: 3-8.*
[20]. Palama TL, Menard P, Fock I, Choi YH, Bourdon E, Govinden-Soulange J, Bahut M, Payet B, Verpoorte R, & Kodja H. 2010. Shoot differentiation from Protocorm Callus Cultures of *Vanilla planifolia* (Orchidaceae): Proteomic and Metabolic Responses at Early Stage. *BMC Plant Biology* 10:82.
[21]. Rajeev P & Dinesh R. 2005. *Vanilla* (Extension Pamphlet). VA. Parthasarathy, Indian Institute of Spices Research. Printers Castle. Kochi. *Euphytica* 96: 237 – 245.
[22]. Remotti PC, Löfler HJM & Loten-Doting LV. 1997. Selection of Cell Lines and Regeneration of Plants Resistance to Fusaric Acid from Gladiolus x grandiflorus cv. 'Peter Pear'. Ruzin SE. 1999. *Plant Microtechnique and Microscopy*. Oxford University Press. New York. 307 p.
[23]. Sticher L, Mauch-Mani B & Metraux JP. 1997. Systemic Acquired Resistance. *Annual Review Phytopathology* 35: 235-270.
[24]. Toyoda H, Hayashi H, & Yamamoto K. 1984. Selection of Resistance Tomato Calli to Fusaric Acid. *Ann. Phytophatol. Soc. Japan.* 50: 538-540.
[25]. Umamaheswari R & Mohanan KV. 2011. A Study of the Asociation of Agronomic Characters in *Vanilla planifolia* Andrews. *International Journal of Plant Breeding and Genetics* 5 (1): 53-58.

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