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DNA PATTERN ANALYSIS OF *Vanilla planifolia* Andrews PLANTLET WHICH RESISTANT TO *Fussarium oxysporum* f. sp. *vanillae*

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ABSTRACT

Stem rot disease is one of the production constraints in vanilla cultivation (*Vanilla planifolia* Andrews) caused by *Fusarium oxysporum* f. sp. *vanillae* (*Fov*). This disease has not been effectively addressed even though several studies have been conducted. Important disease control alternatives to vanilla include the use of *Fov* resistant cultivars. The *Fov* resistant vanilla planlet has been selected in vitro in Murashige & Skoog (MS) medium with the addition of Fusaric Acid (FA) at concentrations of 0, 90, 100, 110, and 120 ppm.The FA tolerant concentration for plantlet selection with vanilla steady growth is between 90 ppm-110 ppm; the 110 ppm of FA was effective for suppressing the *Fov* compared to 90 ppm and 100 ppm respectively. To obtain an overview of the mechanism of resilience of planlet to *Fov*, a more in-depth study of the pattern of DNA *V. planifolia* planlet resistant to *Fov* compared with control. The DNA pattern analysis using PCR_RAPD method. The results showed that a new (specific) band of DNA in a resistant plantlet in size of 930 bp (OPB_14), 430 bp (OPB_20), 230 bp and 270 bp (OPD_19) respectively. These bands predicted as a candidate marker RAPD which responsible for vanilla resistant to *Fov*. A new (specific) band of DNA can become a specific grouping and separate character to vanilla plantlet control and that to induce with FA of 90, 100, 110 ppm.

KEYWORDS: *Vanilla planifolia*, The foot rot disease, *Fusarium oxysporum* f.sp. *vanillae*, in vitro, Fusaric Acid.

INTRODUCTION

Vanilla is one of export commodities industry in Indonesia foreign exchange. *United Nations Development Program (UNDP),* recommends that vanilla Indonesia is no different from "Bourbon vanilla" which has a very good commodity image in the international community.[1,2] The genus of vanilla consists of about 150 species, but the only economical value is 3 species namely *V. planifolia* Andrews, *V. tahitensis* J. Wi Moore and *V. pompona* Schieda.[3,4,2] The most widely cultivated species, especially in Indonesia is *V. planifolia* Andrews.[5,6,7]

The foot rot disease on vanilla is the most vital disease caused by *Fusarium oxysporum* f. sp. *vanillae* (*Fov*), which is of the one constraint and up to know days is not well yet managed. One an alternative way to control foot rot disease could be done by using a cultivar, which was resistant to that disease. In order to get the new cultivar, which is resistant to *Fov* by using an *in vitro* selection method on medium containing fusaric acid.[8] Fusaric acid (FA) is a metabolite produced by several fungal species of the *Fusarium* genus. Fusaric acid may be toxic (concentrations greater than 10^{-5} M) which inhibit the oxidation of cytokines and respiration processes in mitochondria, decrease *Adenosin Tri Phosphat* (ATP) in plasma membranes and reduce polyphenol activity inhibiting growth and regeneration of cultures,[9,8] but at non-toxic concentrations (below 10^{-6} M) actually helps to induce the synthesis of phytoalexin, a form of plant response to inhibit pathogen activity.[8] The use of FA as a selecting agent in in vitro selection may produce insensitive cells or mutant tissues against FA, so that once regenerated into plants may produce resistant strains of pathogenic infection.[8] Vanilla plantlet regeneration in vitro is necessary to precede the selection process with FA, by using plant vegetative parts (explant) in an artificial medium containing growth regulators under aseptic conditions. In plants treated with FA, it will activate genes such as peroxidase gene.[10] Identification of gene products produced during vanilla planlets selected by FA, can be detected by molecular markers.[11,12]

Induced mutations have been widely used in the extension of genetic diversity and improvement of phenotypic characters to increase the production and quality of plants.[13] Increased genetic diversity or plant breeding quality with mutagen has been widely reported in various plants. Genetic diversity resulting from mutations can be detected by molecular markers.[14] Molecular markers are widely used in the activities of genetic diversity analysis, one of which is Random Amplified Polymorphic DNA (RAPD). This marker is based on Polymerase Chain Reaction (PCR) which has been widely used in research with 10 random primary primers.[11,12] The RAPD technique has advantages over other molecular marker techniques, such as: the required DNA quantity is slightly 0.5-50.0 ng, does not require radioisotope, and is simpler to the preparation process compared to the Restriction Fragment Length Polymorphism (RFLP) method.[15] The goals of this research were for obtaining a candidate vanilla plantlets were are resistant to *Fov* with to analyze the DNA pattern of resistant vanilla plantlets compared to the control.

MATERIAL AND METHODS

This research was conducted in the in vitro Laboratory, Department of Biology, Faculty of Mathematics and Natural of Sciences, University of Lampung; Plant Structure and Development Laboratory (in vitro research room), Faculty of Biology, Gadjah Mada University; Agricultural Mycology Laboratory, Faculty of Agriculture, Gadjah Mada University; Laboratory of Genetic Engineering, Center for Biotechnology Studies, Gadjah Mada University, Yogyakarta, Indonesia.

Materials

The research material is a vanilla planlet (*Vanilla planifolia*) sterile in a culture bottle obtained from Regional Technical Implementation Unit; Agriculture, Plantation and Forestry Department, Magelang Regency, Central Java, Indonesia. The tools for DNA analysis include mortar, pestle, gloves, microtube size 0.2 mL and 1.5 mL, centrifuge machine, micropipette, yellow and white tip, ice box, analytical scale, shaker, electrophoresis tank, spectrophotometer (Beckman, DU-65), hot plate, microwave, PCR machine (GeneAmp 2400), UV transiluminator, and Canon Ixus 951S digital camera. The chemicals for DNA isolation are the Nucleon-Phytopure RPN-8511 (Reagent Phytopure I, Reagent Phytopure II, and Resin Phytopure), cold isopropanol, 70% chloroform, cold chloroform. The chemicals used for Polymerase Chain Reaction (PCR) include three RAPD primers (OPB_14, OPB_20, OPD_19), Kappa2G Fast ReadyMix containing KAPA2G Fast DNA polymerase (0.5 U / 25 reaction), KAPA2G Fast PCR buffer, dNTPs (0.2 mM), MgCl2 (1.5mM), and stabilizer. The chemicals used for DNA electrophoresis are TBE 1x, Good View gel color, agarose gel, and 100 bp DNA marker (Vivantis).

Preparation of Materials

For analysis of DNA pattern, randomly samples were taken on 3 samples of moderate vanilla planlet (90 ppm and 100 ppm) and resistant (110 ppm), and control to 12 samples. The 12 samples are: $V_{0.2}$, $V_{0.4}$, $V_{0.6}$, $V_{90.6}$, $V_{90.8}$, $V_{90.9}$, $V_{100.5}$, $V_{100.12}$, $V_{100.13}$, $V_{110.4}$, $V_{110.8}$, $V_{110.9}$. Each sample was then amplified with RAPD-PCR and the results were analyzed visually.

DNA Isolation of Plantlet Vanilla

DNA using the Nucleon Phytopure RPN-8511 kit.[16] Vanilla plantlet leaves are cut into small pieces with sterile scalpels, then weighed 0.1 g. Leaf sample was then crushed with mortal and pestle, added 500 µL Phytopure I reagent while crushed until soft, then inserted in 1.5 mL tube. Thereafter, 150 µL phytopure II reagents were added into the sample and shaken slowly (shaken by hand). The sample was then incubated at 65 ºC above the waterbath for 10 min, then placed in ice box for 20 min, then put 400 µL chloroform cold and 20 µL Phytopure resin into the sample. The sample was then centrifuged at 3000 rpm for 10 minutes, the supernatant was transferred to a tube of 1.5 mL. Cool isopropanol is added to the same volume as the supernatant volume and shaken slowly. Then the sample at the centrifuge returned at 10,000 rpm for 10 min, the supernatant was discarded and the white pellet DNA was then washed by adding 50 µL of 70% alcohol and at the centrifuge at 10,000 rpm for 5 min. The washing with the 70% alcohol is repeated 3 times. Alcohol scraps 70% removed, then the DNA pellets are dried. Pellet DNA after dry then added TE 50 µL buffer until dissolved and then stored in freezer at -20 ^{0}C.

Testing the Quality of Isolated DNA

DNA samples prior to use in PCR reactions were tested for quality and concentration using GeneQuant (Life Science, Ltd., UK). A total of 2 µL isolated DNA samples were inserted into a quartet containing 1998 µL of sterile Iabides and shaken slowly to homogeneous. The sample DNA concentration was read on the spectrophotometer at wavelengths of 260 and 280 nm. The DNA concentration ratio of samples at each wavelength was used as a measure of DNA quality. According to,[17] DNA is of good quality when it has an A260 / 280 = 1.8 - 2.0 ratio.

Analysis of genetic diversity of vanilla planlet by RAPD method

PCR-RAPD

For PCR analysis, a prepared DNA template was dissolved in the TE, ice box, and primer used (Table 1). Then made a PCR premix with the composition: KAPPA2G Fast ReadyMix kit of 12.5 µL, 2.5 µL primer at 100 µM concentration, 1.0 µL DNA template at concentration 40 ng / µL, and dH2O of 9.0 µL, so the volume The total is 25.00 µL.

Table 1: RAPD Primer.

No	Primer	Nucleotide sequence (5'—3')	Reference
1	OPB_14	TCC GCT CTG G	Minoo *et al*., 2008
2	OPB_20	GGA CCC TTA C	Minoo *et al*., 2008
3	OPD_19	CTG GGG ACT T	Minoo *et al*., 2008

Next the premix is amplified with a PCR machine (GeneAmp 2400). The reaction conditions for the PCR-RAPD process follow the[12] modified (Table 2).

Table 2: PCR-RAPD Reaction Conditions.

Reaction	Temperature (°C)	Time (seconds)	
Predenaturasi	95	180	
Denaturasi	95	15	Cycle : 45 x
Annealing	36	15	
Elongasi	72	30	
Post-elongasi	72	420	

Electrophoresis

Electrophoresis is carried out with the following steps. A 500 mL buffer of TBE1x was prepared by taking 50 mL of a buffer solution of TBE10x, then diluted to a 500 mL measuring cup with aquadest added to a 500 mL mark then homogenized. Minigel agarose 1.5% (g /v) was prepared by 1.5 g of agarose incorporated into Erlenmeyer and 100 mL of TBE1x added and homogenized. Then heated using microwave (t = 100 ºC; ± 2 min) until all dissolved, marked with clear-looking solution. The solution was then cooled to a temperature of approximately 50-55 ºC, then added a good view of 5 µL. The liquid agarose is poured into a glassplate with a perpendicular comb. The gel is waited until it stays for ± 30 minutes and after cool comb is lifted. Furthermore, a 25 µL DNA sample (PCR run) is pipetted and inserted into the wells contained in the gel using a microphone. A total of 10 µL DNA markers were then fed at the wells at the left end of the gel. The gel is then fed into an electrophoresis tank that has been filled 1% TBE buffer (v/v). Furthermore, it runs at 100 volts for about 30 minutes.

Visualization of amplification results

Visualization of the results of electrophoresis running on the gel was done using UV transilluminator and photographed as documentation.

RESULTS AND DISCUSSION

For analysis of DNA pattern, randomly samples were taken on 3 samples of moderate vanilla planlet (90 ppm and 100 ppm) and resistant (110 ppm), and control to 12 samples. The 12 samples are: $V_{0.2}$, $V_{0.4}$,$V_{0.6}$, $V_{90.6}$, $V_{90.8}$, $V_{90.9}$, $V_{100.5}$, $V_{100.12}$, $V_{100.13}$, $V_{110.4}$, $V_{110.8}$, $V_{110.9}$. Furthermore, each sample was amplified with RAPD-PCR and the results were analyzed visually.

The quality and quantity of Vanilla planifolia DNA

Total genomic isolation of DNA of Vanilla plantlet leaves of FA at concentrations of 90, 100, 110 ppm and control was performed for a random sample, using a Nucleon Phytopure RPN-8511 kit. The result is a value of DNA purity and DNA concentration (Table 3).

Table 3: The results of DNA purity measurements and DNA concentrations of 12 samples of vanilla control and induced fusaric acid (90, 100, and 110 ppm).

No	Sample	DNA Purity ($A_{260}/_{280}$)	DNA Concentration (ng/µL)
1	$V_{0.2}$	1,853	594,7
2	$V_{0.4}$	1,843	584,7
3	$V_{0.6}$	1,849	590,8
4	$V_{90.6}$	1,869	709,7
5	$V_{90.8}$	1,874	714,2
6	$V_{90.9}$	1,876	716,4
7	$V_{100.5}$	2,000	740,7
8	$V_{100.12}$	1,998	737,5
9	$V_{100.13}$	2,000	736,6
10	$V_{110.4}$	1,869	118,1
11	$V_{110.8}$	1,864	113,5
12	$V_{110.9}$	1,871	119,9

Information:

1-3= Vanilla planlet control ($V_{0.2}$, $V_{0.4}$,$V_{0.6}$)
4-6= Vanilla planlet is induced with FA concentration of 90 ppm ($V_{90.6}$, $V_{90.8}$, $V_{90.9}$)
7-9= Vanilla planlet is induced with FA concentration of 100 ppm ($V_{100.5,}$ $V_{100.12}$, $V_{100.13}$)
10-12= Vanilla planlet is induced with FA concentration of 110 ppm ($V_{110.4}$, $V_{110.8,}$ $V_{110.9}$)

The quality and quantity of DNA was measured using a spectrophotometer (Beckman, DU-65). Table 3 shows that the DNA obtained has a relatively good quality and quantity. DNA purity was obtained from the ratio of absorbance of A260 / 280. The purity ratio value of vanilla DNA isolation ranged from 1,843 - 2,000. This is in accordance with the opinion of[17] which states that the value of a good purity ratio of DNA is in the range of 1,800-2,000. DNA concentration of vanilla planlet from all samples obtained between 113.5 - 740.7 ng/µL. The DNA concentration is then diluted with the aim that the amount of DNA used for PCR amplification has the same concentration in order to obtain a uniform amount of DNA amplification.

The RAPD method was developed based on PCR, which has advantages such as requiring relatively fewer DNA quantities of 0.5-50.0 ng[15] or 5.0-25.0 ng[18] in every PCR chain,[19] reported the template DNA concentration used for the identification of *Jatropha curcas* L. access approximately 50 ng /µL in each PCR reaction.[3] and[4] used a template DNA concentration of 20 and 40 ng / µL

in the amplification of vanilla plant PCR. In this study, the DNA concentrations of all samples of vanilla planlets for templates in PCR reactions were uniformed at approximately 40 ng/µL with dilution.

RAPD Band Analysis

Based on the amplification of DNA sequences using 3 primers on 12 samples of Vanilla plantlets (control) and induced Fusaric acid plantlet (concentrations of 90,100 and 110 ppm) yielded a total of 20 DNA bands. The number of PCR-RAPD amplified bands of the 12 samples of Vanilla planlets is presented in Table 4.

Table 4: The amount of PCR-RAPD amplified bands on control vanilla plantlet and induced fusaric acid (90, 100, and 110 ppm).

No	Primer	Bases Nucleotide Sequence (5'-3')	Number of RAPD Bands	Number of Polymorphic Bands	Number of Mono Bands Morphic	RAPD Band Size(Bp)
1	OPB_14	TCC GCT CTG G	10	01	09	160 – 930
2	OPB_20	GGA CCC TTA C	06	01	05	180 – 430
3	OPD_19	CTG GGG ACT T	04	02	02	170 – 270
		Total	20	04	16	160 – 930

Information
bp: *base pair*
band= fragmen

Table 4 shows that overall, the amplification of 3 primers (OPB_14, OPB_20, and OPD_19) resulted in the number of DNA bands of 4 (OPD_19), 6 (OPB_20), and 10 (OPB_14) per primer on DNA band size between 160 bp to 930 bp . Primers that produce the least number of DNA bands are primer OPD_19 (4 bands) and the most are primers OPB_14 (10 DNA bands). The amount of DNA bands produced depends on how primers recognize their complementary DNA sequences in their template DNA.[20]

In this study the primary selection was based on a study conducted by[4] that examined genetic variation and kinship relationships in the *Vanilla planifolia* species. Of the 9 primers used in the study, 3 primers produced 100% polymorphic DNA bands of primers OPB_14 (6 DNA bands), OPB_20 (8 DNA bands), and OPD_19 (8 DNA bands), a total of 22 DNA bands. Based on these studies may be conserve sequences (fragments of DNA which always appear many times in the DNA genome) in vanilla, more accommodating amplification with these three primers. Therefore, research on Vanilla planlet also uses the same 3 primers, so it is expected to give relatively quick results. The results of Vanilla planlets (control) and induced Fusaric acid plantlet (concentrations of 90, 100, and 110 ppm), using the same primer, yielded 20 DNA bands, OPB_14 (10 DNA bands), OPB_20 (6 DNA bands) And OPD_19 (4 DNA bands).

Electrophoresis result of PCR-RAPD amplification with 3 primers yield 6 patterns of DNA band. The pattern of non-controlled and controlled DNA vanilla bands of FA (concentrations of 90, 100, and 110 ppm) in each of the primers is presented in Tables 5, 6, and 7.

Primer OPB_14

Primer OPB_14 produces two patterns of DNA bands as presented in Table 5.

Table 5 shows the DNA bands of 160, 230, 280, 310, 340, 380, 430, 590, and 680 bp formed on all samples both in the control and in the FA induction. In planlets induced with FA (concentrations of 90, 100, and 110 bp) there is a new (specific) DNA band of 930 bp. So there is a new band on vanilla planlet which in all FA induction concentration.

Table 5: Pattern of DNA band of controlled Vanilla planlet and the induction of fusaric acid (90, 100, and 110 ppm) with primer OPB_14.

DNA-1 band pattern		DNA-2 band pattern	
Sample	**DNA size (bp)**	**Sample**	**DNA size (bp)**
	160		160
	230		230
	280		280
	310	$\mathbf{V_{90.6}, V_{90.8}, V_{90.9}}$	310
$\mathbf{V_{0.2}, V_{0.4}, V_{0.6}}$	340	$\mathbf{V_{100.5}, V_{100.12}, V_{100.13}}$	340
	380	$\mathbf{V_{110.4}, V_{110.8}, V_{110.9}}$	380
	430		430
	590		590
	680		680
			930*

Information: The DNA-1 band pattern: V_0 (control) produces 9 identical bands; The DNA-2 band pattern: V_{90}, V_{100}, V_{110} produces 10 identical bands, and 9 of which are the same as the controls, and 1 new band (*)

Primer OPB_20

Primer OPB_20 produces two patterns of DNA bands as presented in Table 6.

Table 6: Pattern of DNA band of controlled Vanilla planlet and the induction of fusaric acid (90, 100, and 110 ppm) with primer OPB_20.

DNA-1 band pattern		DNA-2 band pattern	
Sample	**DNA size (bp)**	**Sample**	**DNA size (bp)**
	180		180
	220	$V_{90.6}$, $V_{90.8}$, $V_{90.9}$	220
$V_{0.2}$, $V_{0.4}$,$V_{0.6}$	240	$V_{100.5}$, $V_{100.12}$,$V_{100.13}$	240
	280	$V_{110.4}$, $V_{110.8}$, $V_{110.9}$	280
	320		320
			430*

Information: The DNA-1 band pattern: V_0 (control) produces 5 identical bands; The DNA-2 band pattern:V_{90}, V_{100}, V_{110} produces 6 identical bands, and 5 of them are the same as controls, as well as 1 new band(*)

Table 6 shows the DNA bands of 180, 220, 240, 280, and 320 bp formed on all samples both on the control and in the FA induction. In planlets induced with FA (concentrations of 90, 100, and 110 bp) there is a new (specific) DNA band of 430 bp. So there is a new band on Vanilla planlet which in all FA induction concentration.

Primer OPD_19

Two DNA banding patterns were generated using OPD_19 primers as presented in Table 7. In Table 7 the 170 and 200 bp DNA bands were formed on all samples in both the control Vanilla plantlet and the induced fusaric acid. In the fusaric acid-induced plantlets (concentrations of 90, 100, and 110 bp) there are new (specific) DNA bands of 230 and 270 bp. So there are 2 new bands on the Vanilla planlet which in fusaric acid induces all concentrations.

Based on Tables 5, 6, and 7 it can be seen that the RAPD 3 primer application used produces 6 patterns of DNA bands. According to[21] and[22] RAPD essentially utilizes different PCR amplification patterns caused by differences in the position of primer attachment to the genomes of different individuals. The occurrence of differences in ribbon pattern is due to the process of amplification of DNA strands at certain positions.

Table 7: Pattern of DNA band of controlled Vanilla planlet and the induction of fusaric acid (90, 100, and 110 ppm) with primer OPD_19.

DNA-1 band pattern		DNA-2 band pattern	
Sample	**DNA size (bp)**	**Sample**	**DNA size (bp)**
$V_{0.2}$, $V_{0.4}$,$V_{0.6}$	170	$V_{90.6}$, $V_{90.8}$, $V_{90.9}$	170
	200	$V_{100.5}$, $V_{100.12}$,$V_{100.13}$	200
		$V_{110.4}$, $V_{110.8}$, $V_{110.9}$	**230***
			270*

Information: The DNA-1 band pattern: V_0 (control) produces two identical bands; The DNA-2 band pattern: V_{90}, V_{100}, V_{110} produces 4 identical bands, 2 of which are the same as controls, and 2 new / specific bands(*)

Based on the 6 DNA bands patterns, there were four new (930 bp) DNA bands (OPB_14 primer), 430 bp (OPB_20 primer), 230 bp and 270 bp (OPD_19 primer). The specific DNA bands can be used as a character for grouping and separating the control and in fusaric acid induction Vanilla plantlets (concentrations 90, 100, 110 ppm). The appearance of the DNA band pattern and the specific new band on each primer is presented in Figure 1. The results of DNA amplification of Vanilla planlets using primers of OPB_14, OPB_20 and OPB_19, showed that the pattern of DNA bands of control Vanilla planlets was different from the pattern of DNA bands of Vanilla planlets induced with fusaric acid (concentrations 90, 100, and 110 ppm) (Figure 1A,1B, and 1C). The fusaric acid vanilla planlet of either 90, 100 or 110 ppm concentrations yields 1 DNA bands of different size with Vanilla control planlets, 930 bp (OPB_14) and 430 bp (OPB_20) DNA bands, so that these DNA bands can be made as a distinction between Vanilla control planlet and vanilla planlet in induction of fusaric acid (concentrations of 90, 100, and 110 ppm). The DNA band is named as RAPD marker OPB_14_{930} and RAPD OPB_20_{430}. For primers OPD_19 produces a new band of 230 bp and 270 bp, these DNA bands are named as RAPD markers OPD_19_{230} and RAPD markers OPD_19_{270}.

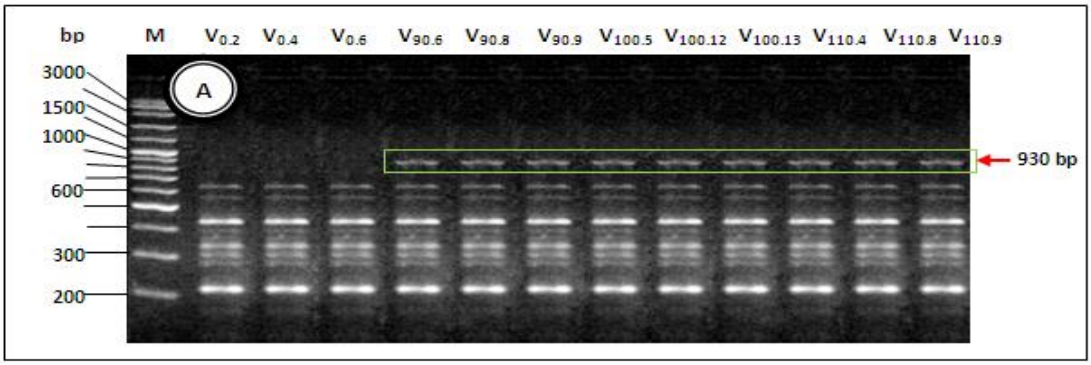

Figure 1(A): Pattern of DNA band of *V. planifolia* planlet with primer OPB_14, yielding new band (specific) 930 bp.

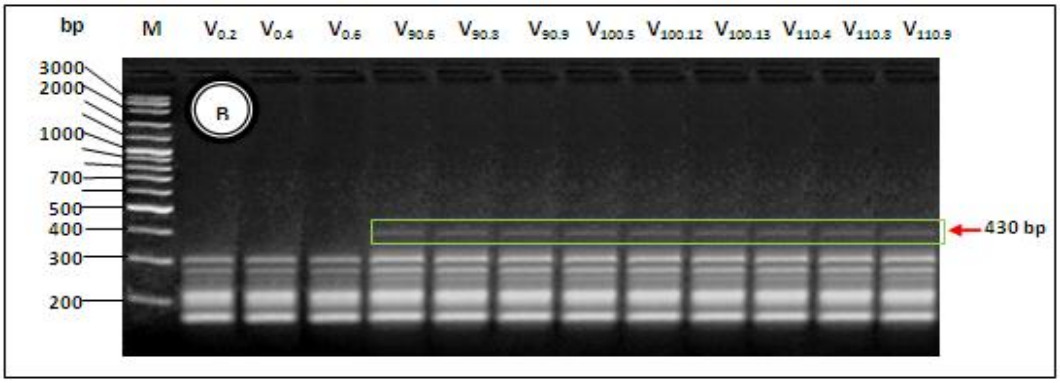

Figure 1(B): Pattern of DNA band of *V. planifolia* plantlet with primer OPB_20, yielding new band (specific) 430 bp.

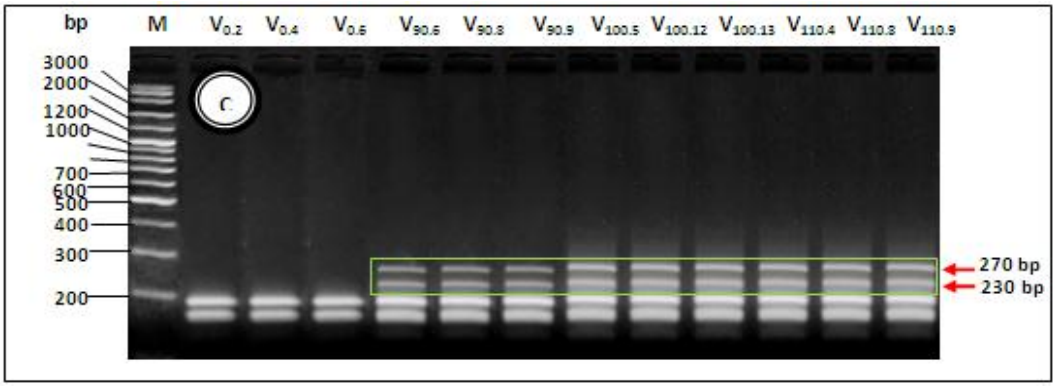

Figure 1(C): Pattern of DNA band of *V. planifolia* plantlet with primer OPD_19, yielding new band (specific) 270 bp and 230 bp.

Based on RAPD markers found, RAPD markers can differentiate controlled vanilla controls that are susceptible to *Fov* with induced planlets with fusaric acid at concentrations of 90, 100, and 110 ppm. The induced vanilla planlet with fusaric acid at concentrations of 90, 100, and 110 ppm, based on the *Fov* resistance test, is a vanilla planlet with moderate and resistant criteria.

The results of this study are supported by,[23] which suggests that RAPD markers can distinguish between varieties of tea plant germplasm collection in China. In cotton plants, RAPD markers can be used to categorize diploid and tetraploid cotton cultivars.[24,25] States that RAPD markers RAPD UBC_218$_{768}$, UBC_218$_{602}$, dan UBC_237$_{248}$, can be used to distinguish between *Eucalyptus globulus* that is resistant to cold (frozen) temperatures, with sensitive plants.

Next,[26] suggests that RAPD markers can be used to distinguish the Carnation planlet (*Dianthus caryophylus* L.) resistant to *Fusarium oxysporum* f. sp. *dianthi* and planlets that are sensitive to the fungus in vitro.[27] has been able to distinguish pinang planlet (*Areca catechu* L.) resistant to *Yellow Leaf Disease* (YLD) disease with in vitro sensitive plantlets.[28] and[29] used RAPD to evaluate the genetic stability of the Apricot (*Prunus armeniaca* L.) planet and *Kaempferia galanga*, micropropagation results in vitro compared to the parent.

Based on the above description, it can be stated that the Vanilla plantlet control (vulnerable) is genetically different from the vanilla planlet induced with fusaric acid at concentrations of 90, 100, and 110 ppm (moderate and resistant) to *Fusarium oxysporum* f.sp. *vanillae*, the cause of vanilla stem rot disease.

All DNA template *V. planifolia* tested, it can be amplified with primers OPB_14, OPB_20, and OPD_19. The PCR DNA amplification results show that moderate and resistant vanilla planlets at FA 90, 100, and 110 ppm form new DNA bands. These specific bands are indicative of the identification of the new cultivars of vanilla resistant planlet *Fov*. Thus, DNA bands of 930 bp (OPB_14), 430 bp (OPB_20), as well as 230 bp and 270 bp (OPD_19), can be predicted as RAPD marker candidates for vanilla plantlet resistance to *Fov*.

CONCLUSION

Vanilla resistant plantlet character resistant to *Fusarium oxysporum* f. sp. *vanillae* can be proven molecularly by means of DNA pattern analysis. New (specific) DNA bands vary in size depending on the primer used. Specific DNA bands of 930 bp (OPB_14), 430 bp (OPB_20), as well as 230 bp and 270 bp (OPD_19), can be predicted as RAPD marker candidates for the resistance of the *Vanilla planifolia* planlet to *Fusarium oxysporum* f.sp. *vanillae*. The specific DNA bands can be used as characters to group and separate vanilla

control planlets and induced with fusaric acid (concentrations of 90, 100, 110 ppm).

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