

CVPD DNA FRAGMENT AFFECT DIFFERENCES IN RESISTANT TO CITRUS VEIN PHLOEM DEGENERATION (CVPD) DISEASE, NUTRIENT DEFICIENCIES AND QUALITY OF FRUITS

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CVPD^f DNA FRAGMENT AFFECT DIFFERENCES IN RESISTANT TO CITRUS VEIN PHLOEM DEGENERATION (CVPD) DISEASE, NUTRIENT DEFICIENCIES AND QUALITY OF FRUITS

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ABSTRACT

Citrus Vein Phloem Degeneration (CVPD) disease is a major obstacle in the effort to develop and increase the production of citrus fruits in Bali. The study on the polymorphism of CVPD^f DNA fragment shows that the CVPD^f DNA fragment is resistant factor against CVPD disease. This study try to elaborate the difference in resistance led to differences in plant nutrients deficiencies in the citrus plant with CVPD disease. . Besides, there are also difference in the quality of fruit due to CVPD disease attacks such as water content, vitamin C content and antioxidants in citrus fruits, color, flavor, taste and texture and fruit into small, hard and sour taste.

Keywords: CVPD^f DNA Fragmen, CVPD, citrus, nitrient deficiency

INTRODUCTION

Citrus plants (*Citrus spp.*) is one of the most important horticultural crops in the community economy. This plant has long been known and cultivated in Indonesia (Wahyuningsih, 2009). Citrus is a local fruit that is quite a lot on the market, the quality of citrus fruit can be influenced by various factors such as Citrus Vein Phloem

Degeneration (CVPD) (Wirawan *et al.*, 2014, Wirawan *et al.*, 2017).

CVPD disease is one of the most important diseases of citrus crop that has grown and become the main obstacle to the development and improvement of citrus production in Bali (Wijaya, 2007). CVPD disease causes the quality of the fruit to be small, it's sour that many disadvantage farmers. CVPD disease is caused by the

bacterium *Libeobacter asiaticus*, the Leaf analysis is a method of bacteria enter the plant cells through the estimating nutrient needs based on the insect puncture vector *Diaphorina citri*, it assumption that within certain boundaries causes leaves of yellowing plants there is a positive relationship pattern (chlorosis) and fruit small (Wirawan *et al.*, between nutrient availability, leaf nutrient content, and fruit yield and quality 2004).

CVPD^f DNA fragment (Wirawan *et al.*, 2004, Wirawan, *et al.*, 2016) plays a role in the resilience of citrus plants against CVPD disease. The CVPD^f DNA fragment is found in citrus crops that are resistant or relatively resistant to CVPD disease, namely *Triphacia trifoliata* and *Citrus aurantifolia* var seedless. However, this CVPD^f DNA fragment is also found in the citrus plants that are susceptible to CVPD disease, this is due to the presence of polymorphism in CVPD^f DNA fragments (Yuniti, 2017). This polymorphism difference causes the difference of citrus plant resistance to the pathogen of CVPD disease.

In this research will be analyzed nutrient deficiency among the citrus plants

with CVPD disease which have the polymorphism difference in CVPD^f DNA fragments. Fruit qualities were also analyzed that were included vitamin C content, antioxidant content, water content, fruit size, texture, aroma, and the color of fruit.

MATERIALS AND METHODS

This research was conducted in several stages starting with sample collection, in this study using *Citrus nobilis*, *Citrus reticulata* var selayar, *Citrus reticulata* var keprok taken at citrus plantation centre in Bali, then analyzed nutrient deficiency.

Plant tissue analysis was performed to determine the nutrient status of plants, with Atomic Absorbance Spectrophotometer (AAS) method based on absorption of light by atoms. The study also tested organoleptics on color, aroma, texture and size, determining moisture content by using oven method (AOAC, 1995), vitamin C analysis with Iodine test, and antioxidant activity test by DPPH method.

RESULTS AND DISCUSSIONS

The Yuniti research (2017), showed that CVPD^f DNA fragments indicated an important role in orange plant resistance to CVPD disease.

Table 1: Resistance and sensitive orange with CVPD

No	Resisten	Sensitif
1	<i>T. trifoliata</i>	<i>Citrus aurantifolio</i>
2	<i>C. aurantifolia</i> var seedles	<i>Citrus amblycarfa</i>
3		<i>Citrus nobilis</i>
4		<i>Citrus reticulata</i>
5		<i>Citrus grandis</i>

Citrus grown like *Citrus nobilis*, This difference in resistance causes *Citrus reticulata* var selayar, *Citrus* differences in plant deficiencies to *reticulata* var keprok citrus which is nutrients. Based on the visual symptoms of susceptible to CVPD disease in fact contain nutrient balance is an important factor in CVPD^r fragments are genes indicated to describing the symptoms of deficiency and provide resistance to CVPD disease in caused by more than one particular nutrient. citrus plants such as *Triphasia trifoliata* Samples showing CVPD symptoms did not and *Citrus aurantifolia* var seedless all yield DNA bands of *L. asiaticus* bacteria containing CVPD^r gene so it becomes but from all samples showed a nutrient difficult to explain the mechanism of CVPD deficiency of certain minerals (Wirawan, *et al.*, 2017). This means that the lack of disease attacks against citrus crops. Table 1. nutrients on the soil and causing soil shows that resistant *T. trifoliata* and *C.* fertility decreased so that nutrient needs *aurantifolia* var seedless have resistance to required citrus crops are not met then the CVPD, but the cultured citrus that also occurrence of deficiency or by infection of contains CVPD^r DNA fragments is other organisms, as shown in table 2. susceptible to CVPD disease.

Table 2: Results of nutrient analysis with Atomic Absorption Spectrophotometer (AAS) methods

Sample of leaf citrus	Nutrient concentration (%)			
	Zn	Fe	Mg	Mn
Without symptoms	8,86*	70,49	131,10	11,92*
Light symptoms	5,95*	67,11	88,30	4,60*
Medium symptoms	0	66,92	78,35	0
Heavy symptoms	0	65,32	55,23	0

Note: (*) Critical nutrient

Table 2. shows the symptomless and the low to high category. Fe and Mg elements lightly symptomatic citrus plants indicate a for all samples showed nutrient adequacy in deficiency of the Zn nutrients, but on the the plant. Fe element required citrus plants other hand the moderate and heavy orange ranging from 25-200%. Lack of Mn grains are not detected in the presence of element in citrus plants will cause the the Zn element. Zn deficiency for plants growth of dwarf plants, because Mn role in will cause the color of yellow spots on the formation of chlorophyll, helping the young leaves and growth will be stopped process of photosynthesis and stimulate and leaves falling so that it can lead to seed germination and ripening fruit. death. According to Muhammad and Symptoms that can be seen in citrus plants Idaryani (2005) if the micro element when nutrient deficiency is a symptom content exceeds the optimum phase then the abnormalities leaf appears on all plants, plant will experience excessive phases and whereas the CVPD disease attack only on toxic phases. The Zn element of certain plant blocks. (Sudarmi, 2013).

concentration must be present in citrus Based on the visual symptoms of plants between 25-100%. The content of nutrient balance is an important factor in Mg has a range of 55 - 131%, according to describing the symptoms of deficiency and McLaren and Cameron (2005), the Mg caused by more than one particular nutrient. content in plant tissues is usually between From the PCR yield of citrus leaf samples 0.25-6.0%, but research in Malaysia showing CVPD symptoms, not all of them contains higher Mg. which ranged from produced DNA bands of *L. asiaticus* 0.55 to 0.70%. Mg content is included in bacteria but from all samples indicated a

nutrient deficiency of certain minerals, plants infected with CVPD, in order for the these results were in accordance with the protein to function properly, the proteins opinion of Wirawan *et al.* (2017). This means that act as transport proteins will bind to that the lack of nutrients available to citrus specific protein compounds. According to crops will result in the nutrient needs that Toha (2001) a compound to be transpired citrus crops need are not met then there will must first bond with a specific transport protein so that the compound will be able to be nutrient deficiency or by other organism infections. carry through the membrane.

As a result of CVPD disease attacks, The presence of symptoms of CVPD the plant cell ion transport proteins will be disease causes the resulting quality of citrus disrupted resulting in plant cells lacking fruit is also different content of vitamin C, certain minerals or nutrients (Setiawan and water content, antioxidants, flavor, aroma Trisnawati, 1999, Sritamin, 2007). In citrus and color of citrus fruits.

Table 3: Water content, Vitamin C and Antioxidants

No	Sample	Water content (%bb)	Vitamin C (mg/100gr)	Antioxidant (mg/L GAEAC)
1	<i>C. nobilis</i> with CVPD	75,6117	10,925	113,6
2	<i>C. nobilis</i> healthy condition	87,6924	29,265	145,0
3	<i>C. reticulata</i> var selayar with CVPD	79,0115	11,862	107,3
4	<i>C. reticulata</i> var selayar healthy condition	90,4226	31,618	149,9

The results of the study in Table 3 vitamin C and antioxidants differ between show that the content of water content, healthy and sick citrus due to inhibition of

macro or micro nutrient absorption required by plants, lack of root absorption in absorbing nutrients can lead to deficiency of Fe, Zn, Mg and Mn will cause symptoms of chlorosis in plant leaves (Zekri and Obreza, 2012). The symptoms of CVPD attacks also cause the fruit to become small, hard and the color is yellow green.

citrus and healthy plants, performed by measuring the circle of citrus fruit and the weight of citrus fruits suffering from mild, moderate and severe CVPD disease. In Fig. 1. citrus fruit samples were taken each of 5 pieces to weigh the weight and circumference of the fruit, then taken the average weight and circumference of the

The study obtained different citrus fruit, the results as shown in Table 4.

measurements between CVPD-infected

Table 4 Measurement of Citrus Fruits

Sample	Fruit weight (g)	Fruit circumference (cm)
A	216,20	25,70
B	172,41	23,05
C	146,84	22,55
D	124,24	21,40
E	105,66	20,05
F	99,10	19,50
G	60,29	16,40
H	40,37	14,45
I	25,84	12,25

Note:

A-B. Fruit from Healthy Citrus Plants (seen from Physical Plants),
C-D. The Fruit of an Affected Orange Plant A mild CVPD disease,
E-F. The Fruit of an Affected Orange Plant CVPD's disease is and
G-H-I Fruit from Orange Plant that is Aggressive The severe CVPD disease.

Healthy citrus plants produce fruit Healthy orange has fresh fruit, large size, with a larger weight and fruit circumference fruit rim is also large and high water that weighs 216.20 g. with a fruit content. The more severe the CVPD attack circumference of 25.70 cm, while the sick rate on the citrus plant the smaller the citrus plant has a smaller fruit size, has a quantity and quality of the resulting fruit as small fruit circumference as in Table 4. shown in Fig. 1 and 2.



Fig. 1. Visual Physical Citrus Fruit Infected with CVPD Disease

Description: A-B. Fruit from Healthy Citrus Plants, C-D. Fruit from Orange Crops Infected with mild CVPD disease, E-F. The Fruit of the Infected Citrus Plants Medium CVPD Disease and G-H-I Fruits from the Experienced Citrus Plants The severe CVPD disease.

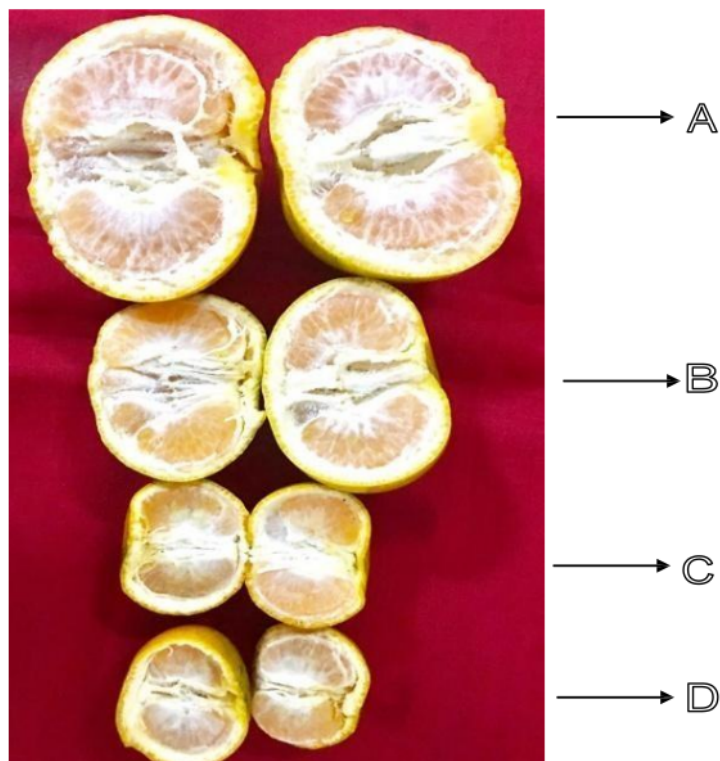


Fig. 2. Differences of healthy citrus fruit and CVPD disease

- A. Citrus fruit is healthy
- B. Citrus fruit attacked lightly
- C. Citrus fruits are attacked moderately
- D. Citrus fruits attacked by weight

The presence of polymorphism differences in citrus crops affects plant resistance to CVPD disease. CVPD disease causes nutrient deficiency, especially Mn and causes the quality of fruit to be low water content, low vitamin C, low antioxidants, fruit color becomes yellowish green, hard texture and taste sour.

The result of this study showed that the difference in the polymorphism of CVPD^r DNA fragment caused the difference in nutrient deficiencies and the quality of the citrus fruits.

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