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

I Gusti Ayu Diah Yuniti, I Gede Putu Wirawan, I NyomanWijaya, Made Sritamin*

Department of Agrotechnology Faculty of Agriculture, Udayana University
**Corresponding author: diah.yuniti@unr.ac.id*

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^r DNA fragment shows that the CVPD^r DNA fragment is resistant factor againt 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^r 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). Citrusis 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 bacteria enter the plant cells through the insect puncture vector *Diaphorina citri*, it causes leaves of yellowing plants (chlorosis) and fruit small (Wirawan *et al*., 2004).

CVPDr DNA fragment (Wirawan *et al*, 2004, Wirawan, *et al*, 2016) plays a role in the resilience of citrus plants against CVPD disease. The CVPDr 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 CVPDr DNA fragment is also found in the citrus plants that are susceptible to CVPD disease, this is due to the presence of polymorphism in CVPDr DNA fragments (Yuniti, 2017). This polymorphism difference causes the difference of citrus plant resistance to the pathogen of CVPD disease.

Leaf analysis is a method of estimating nutrient needs based on the assumption that within certain boundaries there is a positive relationship pattern between nutrient availability, leaf nutrient content, and fruit yield and quality (Srivastava and Singh 2004, Srivastava and Alila 2006). The availability of nutrients over a certain period had a positive effect on fruit and production nutrients the following year in direct response to soil nutrient content (Bhargava 2002, Wall 2010). Leaf tissue analysis as a diagnostic tool has been widely performed on annual crops to determine nutrient requirements prior to nutrient disturbance (Obreza *et al*., 2008). Stebbins and Wilder (2003) reported that leaf nutrient concentration can be used as a guide to determine the nutrient status of plants whose patterns are directly related to plant growth and production.

In this research will be analyzed nutrient deficiency among the citrus plants with CVPD disease which have the polymorphism difference in $CVPD^r$ 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 reticulate* var selayar, *Citrus reticulate* 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 Absorbtion 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 $CVPD^r$ 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	*T. trifoliate*	*Citrus aurantifolio*
2	*C. aurantifolia var seedles*	*Citrus amblycarfa*
3		*Citrus nobilis*
4		*Citrus reticulate*
5		*Citrus grandis*

Citrus grown like *Citrus nobilis*, *Citrus reticulate* var selayar, *Citrus reticulate* var keprok citrus which is susceptible to CVPD disease in fact contain CVPD^r fragments are genes indicated to provide resistance to CVPD disease in citrus plants such as *Triphacia trifoliata* and *Citrus aurantifolia* var seedless containing CVPD^r gene so it becomes difficult to explain the mechanism of CVPD disease attacks against citrus crops. Table 1. shows that resistant *T. trifoliate* and *C. aurantifolia* var seedless have resistance to CVPD, but the cultured citrus that also contains CVPD^r DNA fragments is susceptible to CVPD disease.

This difference in resistance causes differences in plant deficiencies to nutrients. Based on the visual symptoms of nutrient balance is an important factor in describing the symptoms of deficiency and caused by more than one particular nutrient. Samples showing CVPD symptoms did not all yield DNA bands of *L. asiaticus* bacteria but from all samples showed a nutrient deficiency of certain minerals (Wirawan, *et al.*, 2017). This means that the lack of nutrients on the soil and causing soil fertility decreased so that nutrient needs required citrus crops are not met then the occurrence of deficiency or by infection of other organisms, as shown in table 2.

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

Sample of leaf citrus	Nutrient consentration (%)			
	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 lightly symptomatic citrus plants indicate a deficiency of the Zn nutrients, but on the other hand the moderate and heavy orange grains are not detected in the presence of the Zn element. Zn deficiency for plants will cause the color of yellow spots on young leaves and growth will be stopped and leaves falling so that it can lead to death. According to Muhammad and Idaryani (2005) if the micro element content exceeds the optimum phase then the plant will experience excessive phases and toxic phases. The Zn element of concentration must be present in citrus plants between 25-100%. The content of Mg has a range of 55 - 131%, according to McLaren and Cameron (2005), the Mg content in plant tissues is usually between 0.25-6.0%, but research in Malaysia contains higher Mg. which ranged from 0.55 to 0.70%. Mg content is included in the low to high category. Fe and Mg elements for all samples showed nutrient adequacy in the plant. Fe element required citrus plants ranging from 25-200%. Lack of Mn element in citrus plants will cause the growth of dwarf plants, because Mn role in the formation of chlorophyll, helping the process of photosynthesis and stimulate seed germination and ripening fruit. Symptoms that can be seen in citrus plants when nutrient deficiency is a symptom abnormalities leaf appears on all plants, whereas the CVPD disease attack only on certain plant blocks. (Sudarmi, 2013).

Based on the visual symptoms of nutrient balance is an important factor in describing the symptoms of deficiency and caused by more than one particular nutrient. From the PCR yield of citrus leaf samples showing CVPD symptoms, not all of them produced DNA bands of *L. asiaticus* bacteria but from all samples indicated a nutrient deficiency of certain minerals, these results were in accordance with the opinion of Wirawan *et al*. (2017). This means that the lack of nutrients available to citrus crops will result in the nutrient needs that citrus crops need are not met then there will be nutrient deficiency or by other organism infections.

As a result of CVPD disease attacks, the plant cell ion transport proteins will be disrupted resulting in plant cells lacking certain minerals or nutrients (Setiawan and Trisnawati, 1999, Sritamin, 2007). In citrus plants infected with CVPD, in order for the protein to function properly, the proteins that act as transport proteins will bind to specific protein compounds. According to Toha (2001) a compound to be transpired must first bond with a specific transpot protein so that the compound will be able to carry through the membrane.

The presence of symptoms of CVPD disease causes the resulting quality of citrus fruit is also different content of vitamin C, water content, antioxidants, flavor, aroma 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	*C. nobilis with* CVPD	75,6117	10,925	113,6
2	*C.nobilis* healthy condition	87,6924	29,265	145,0
3	*C. reticulate* var selayar with CVPD	79,0115	11,862	107,3
4	*C. reticulate* var selayar healthy condition	90,4226	31,618	149,9

The results of the study in Table 3 show that the content of water content, vitamin C and antioxidants differ between 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.

The study obtained different citrus measurements between CVPD-infected 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 fruit, the results as shown in Table 4.

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

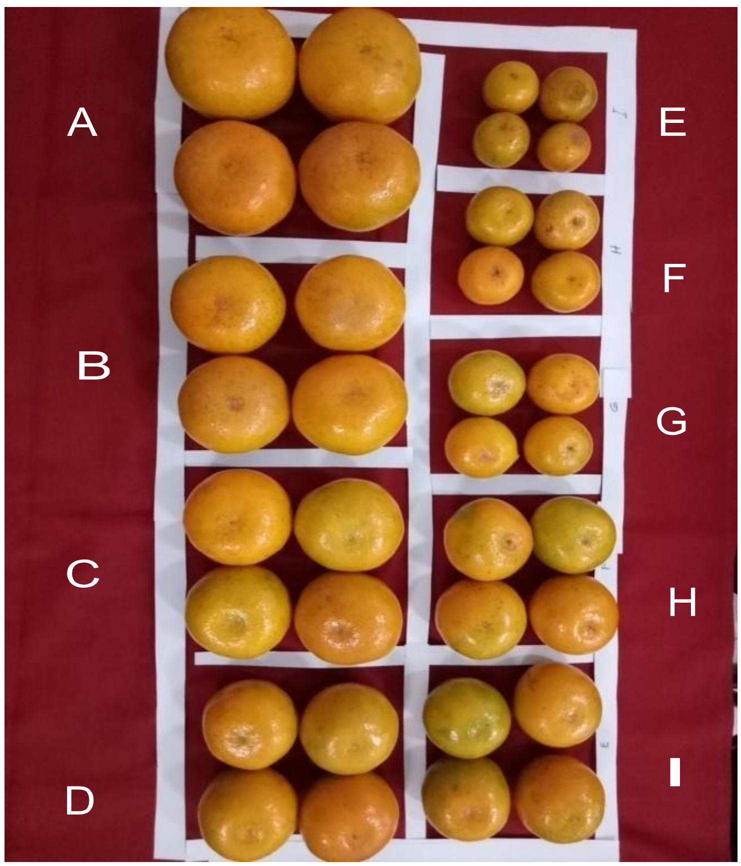

Fig. 1. Visual Physical Citrus Fruit Infected with CVPD Disease
Description: A-B. Fruit from Healthy Citrus Plants, C-D. Fruit from Orange Crops Infected with mild CVPD disease, E-F. The Fruit of the Infected Citrus Plants Medium CVPD Disease and G-H-I Fruits from the Experienced Citrus Plants The severe CVPD disease.

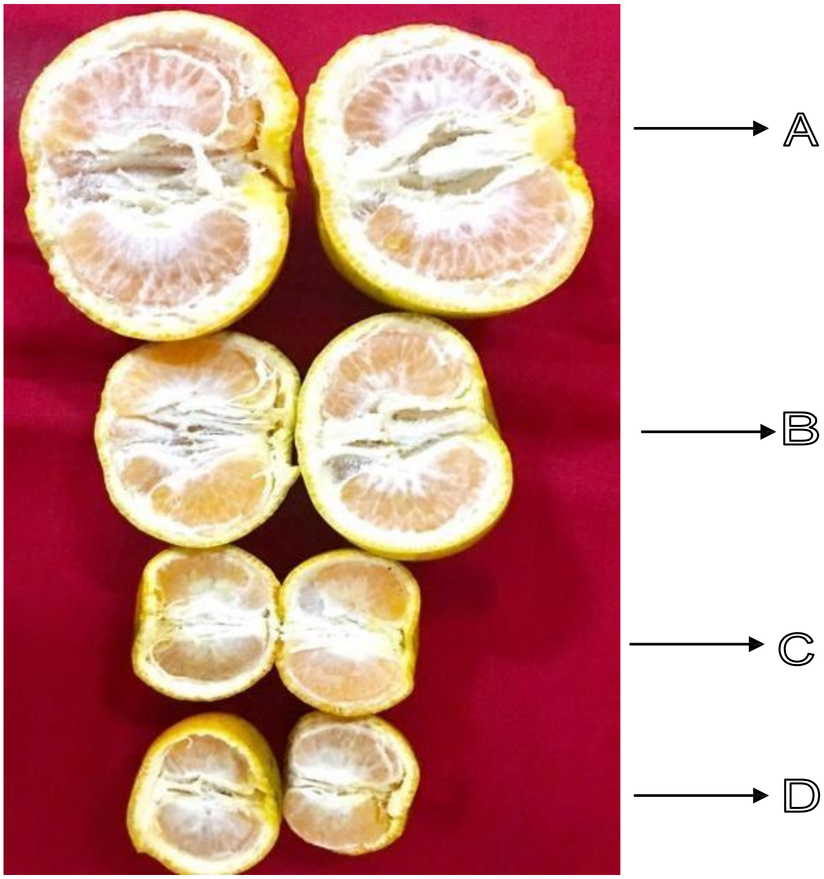

Fig. 2. Differences of healthy citrus fruit and CVPD disease
A. Citrus fruit is healthy
B. Citrus fruit attacked lightly
C. Citrus fruits are attacked moderately
D. Citrus fruits attacked by weight

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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