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The Role of Leaf Nutrients in Vascular Streak Dieback Disease in Cocoa Plants (*Theobroma cacao* L.)

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ABSTRACT

Background: Vascular streak dieback (VSD) is an important disease in cocoa and a decrease in nutrient supply is indicated to increase the severity of this disease. This study aimed to analyze the direct and indirect effects of nutrients that play a role in protecting cocoa plants from VSD.

Methods: The research location was selected based on criteria such as being a smallholder cocoa plantation area, having 5-10-year-old plants and the presence of VSD disease. A sampling technique for observing disease severity was systematically conducted, with the main sample being farmers' plantations. Cocoa leaf samples were taken from branches at one-third of the plant height, specifically from the upper canopy (leaves 3-4), which were physiologically mature, healthy and dark green. The variables observed were disease severity and cocoa leaf nutrient contents. The influence of N, P, K, Mg, Ca, Fe, Mn, B, Cu and Zn on VSD severity was analyzed using path analysis.

Result: The results showed that disease severity was related to nutrient content in the leaves. The cocoa leaf nutrients that have a direct influence on the increase in VSD disease severity are K, Ca, Mn and Cu, while nutrients N, P, Mg, Fe, B and Zn have a direct influence on reducing VSD disease severity. Nutrients N and P have a significant direct influence, whereas nutrient K has a significant indirect influence through its interaction with nutrients N and P in reducing VSD disease severity.

Key words: Cocoa, Nutrient, Path analysis, VSD.

INTRODUCTION

Vascular streak dieback (VSD) disease has recently caused significant losses to cocoa farmers and the severity of the disease continues to increase. The disease is caused by the fungus, *Ceratobasidium theobromae* (Samuels *et al*., 2012). The severity rate of this disease in Southeast Sulawesi is 57.84% (Khaerati *et al*., 2016), whereas in West Sumatra, it ranges from 24.29% to 44.71% (Trisno *et al*., 2016). Production loss due to this disease in susceptible cocoa clones, it can cause plant mortality of up to 59% (Taufik *et al*., 2021).

The increasing severity of the disease over time is associated with environmental and climatic changes (Delgado-Ospina *et al*., 2021). The symptoms of VSD intensify during the dry season because the pathogen attacks the vascular system, leading to impaired water and nutrient supply to the leaves and resulting in branch dieback and leaf shedding (Harni *et al*., 2019). To anticipate these climate changes, plants must always be in a high-vigor condition through a proper and balanced supply of nutrients, enabling them to adapt and withstand environmental stress and pathogen attacks.

Previous studies on VSD control have included the use of biological agents, resistant varieties and fungicide applications (Wahab *et al*., 2016; McMahon *et al*., 2018). However, studies on the interaction between VSD and nutrient availability in cocoa plants at specific locations are limited. Pathogenic fungi that cause the disease depend on plant nutrients to ensure their growth and development through unidirectional nutrient transfer from the plant to the fungus (Filho *et al*., 2021). Therefore, the severity of VSD is suspected to be determined by the presence of nutrients in the host plants, although the details are still limited.

Based on the above description, it is essential to analyze the essential nutrients that play a role in protecting cocoa plants from VSD disease. The role of essential nutrients in plant tissues is crucial for understanding their direct and indirect effects on inhibiting or promoting VSD development. Accurate knowledge of the influence of these nutrients will help determine the planning and formulation of an effective VSD disease control strategy through efficient fertilization. This study was conducted to determine the essential nutrients in plant tissues that directly and indirectly affect the reduction and increase in VSD disease severity in cocoa plants.

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MATERIALS AND METHODS

The method of determining the research location and samples

The research locations were determined based on the criteria of smallholder cocoa plantations, with plant ages between 5-10 years and the presence of VSD disease. The selected locations were the districts of Konawe and Konawe Selatan in Southeast Sulawesi Province, Indonesia. Within each village, to 3-5 cocoa farms were chosen as observation plots. This study was conducted from September 2020 to December 2020.

The sampling technique for disease severity observation and sample determination was performed systematically, with the main sample being the farmer's cocoa plantation. Each cocoa farm unit was divided into sub-plots and plants were observed in a zigzag pattern within the farm, with a total of 10-15 plants. To obtain data on disease severity, the total number of branches and the number of branches showing symptoms are counted.

Cocoa leaf samples were taken from branches at one-third of the plant's height, specifically from the terminal shoots (leaves 3-4) that are physiologically mature and healthy. Four leaves were collected from each plant, one from each cardinal direction (west, east, north and south), resulting in a total of four leaves. Leaves were stored in containers and prepared for shipment to the laboratory.

Observation parameters and data analysis

The variables observed in this study were the severity of VSD disease and leaf nutrient content. The severity of VSD was calculated using the formula (Khaerati *et al*., 2016):

$$DS = \frac{\sum (ni \times vi)}{Z \times N} \times 100\%$$

In which:
DS= Disease severity.
ni= Number of plants showing symptoms at severity level i.
vi= Severity scale value for each attack category, where i= 0, 1, 2, 3, 4.
Z= Highest scale value.
N= Number of observed plants.
The level of attack is categorized as follows:
0= Healthy plants, no symptoms of VSD attack found.
1= $0\% < \times \leq 5\%$ of symptomatic branches with VSD.
2= $5\% < \times \leq 10\%$ of symptomatic branches with VSD.
3= $20\% < \times \leq 50\%$ of symptomatic branches with VSD.
4= $\times > 50\%$ of symptomatic branches with VSD.

The observed parameters of the cocoa leaf nutrient elements were N, P, K, Mg, Ca, Fe, Mn, B, Cu and Zn. Nutrient analysis was conducted at the Molecular and Environmental Laboratory of the Faculty of Sciences at Halu Oleo University. N was extracted through wet digestion using H_2SO_4 and N measurements were performed using an atomic absorption spectrophotometer. The nutrients were determined by wet digestion using a mixture of concentrated acids, HNO_3 and $HClO_4$ and measured using an AAS.

The relationship between the variable of VSD disease severity and the nutrients was analyzed using Pearson correlation analysis. The magnitude of the influence of each nutrient on VSD disease severity and the relationship between variables were analyzed using path analysis with the assistance of Excel and SPSS (IBM Corp). In this analysis, VSD Disease Severity (Y) was the dependent variable and X (each leaf nutrient) was the independent variable.

RESULTS AND DISCUSSION

The severity of VSD disease and the status of nutrient content in cocoa leaves

The severity of VSD in cocoa at the study locations varied from 1.25% (mild) to 95% (severe) (Table 1). The symptoms of VSD observed in the field were yellowing of the second or third leaves from the growing point, with small green spots resembling scattered islands on the entire leaf surface. These leaves eventually fall off, leaving the branches where they are attached bare, as the upper and lower leaves remain intact. When yellowing leaves were plucked from the branches, three brownish spots were observed on the remaining leaf attachment site. If the branch is longitudinally split, brown lines can be observed in the vascular tissue, which is a characteristic symptom of VSD.

Based on Table 1, it is known that cocoa plantations with high disease severity (85-95%) have lower levels of N, P and K than plantations with mild disease severity (1.25-5.63%). The nutrient status of the cocoa was determined based on the criteria proposed by Bahia *et al*. (2021). According to these criteria, 73.33% of cocoa leaf samples contained Nitrogen within the normal to excessive category, Phosphorus: 46.67% are normal, Potassium were deficient in 93.33%, Calcium nutrient is categorized as normal in 73.33% of samples. All samples showed a deficiency in Magnesium. Samples containing Fe within 66.67% are in the excessive category. Based on these data, it can be inferred that the Mn, B, Cu and Zn are deficient or below normal, which is likely to contribute to abnormal plant growth and make them susceptible to pathogenic infections. These data indicate that nutrients play a role in the improvement or decline in plant health. Complete and balanced nutrition serves as the frontline defense of plants against pathogenic infections, thus suppressing disease development (Handrid *et al*., 2022).

Macro-nutrients that showed a highly significant negative correlation with the severity of VSD disease were N, P and K, while other macro-nutrients Ca and Mg exhibited a non-significant negative correlation, indicating a very weak correlation (Table 1). These data suggest that an increase in the availability of the macro-nutrients N, P and K in the leaves is associated with a decrease in the severity of VSD disease. The micronutrient that shows a significant positive correlation with disease severity is Zn, indicating that an increase in Zn is associated with higher disease severity.

The role of leaf mineral nutrition in increasing the severity of vsd disease

The mineral nutrients present in cocoa leaves that have a direct positive influence on the severity of VSD disease (Table 2) are K, Ca, Mn and Cu. These nutrient elements directly contribute to the increase in disease severity, without being influenced by other independent variables (nutrient elements). The presence of these nutrient elements affects the increase in disease severity owing to their deficiency or below-normal status.

The role of K nutrient in increasing disease severity can be reduced by optimizing its indirect effects through other nutrient elements, as indicated by its large negative correlation coefficient (-0.916). The indirect influence of K nutrients through its interaction with N, P, Mg, Fe, Mn, B and Cu nutrients can reduce the severity of VSD in cocoa plants (Table 2). Potassium plays a significant role in plant biochemical and physiological processes, such as enzyme activation, photosynthesis, protein synthesis and enhancing plant resistance to diseases, as well as in improving plant health quality (Oosterhuis *et al*., 2014). Cocoa plants lacking K are susceptible to VSD.

Calcium has a greater direct impact on increasing the severity of VSD than K and Mn. The direct effect of Ca on disease severity can be mitigated by leveraging its indirect effects. Ca indirectly reduces disease severity through its interaction with N, P, Mn, B and Cu. Cocoa plants lacking Ca exhibit fragile cell membranes that can rupture and release intracellular contents, which can serve as food sources for pathogens (Filho *et al*., 2021). The indirect influence of the Mn through its interaction with K, Ca, Fe, B

Table 1: The results of nutrient analysis in cocoa leaves and correlation of nutrients with the severity of VSD disease.

Sample	DS (%)	N (g/kg)	P (g/kg)	K (g/kg)	Ca (g/kg)	Mg (g/kg)	Fe (mg/kg)	Mn (mg/kg)	B (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
LK1	95.00	17.93	0.99	0.11	16.79	0.02	47.83	208.77	0.45	1.08	32.88
LK2	85.00	14.25	0.66	0.22	10.67	0.03	56.97	307.86	1.08	2.28	41.25
LK3	46.88	21.53	1.65	1.87	12.25	0.07	78.55	107.85	0.64	1.97	18.96
LK4	47.50	21.53	2.31	3.99	10.88	0.08	97.32	97.76	0.34	1.86	10.07
LK5	5.63	31.57	2.76	5.10	12.09	0.05	107.86	106.73	0.27	1.94	9.86
LK6	3.13	37.93	2.23	6.96	10.76	0.09	195.62	298.76	0.74	2.76	6.74
LK7	1.25	35.70	1.91	5.13	6.99	0.02	87.55	209.75	0.69	1.85	7.12
LK8	2.50	38.26	2.27	5.10	12.97	0.04	98.26	190.87	0.79	2.07	6.68
LK9	46.25	29.15	2.58	3.23	10.76	0.05	108.95	90.88	0.22	5.22	56.33
LK10	90.63	13.49	0.69	0.68	9.12	0.06	178.54	106.73	0.35	4.12	98.62
LK11	89.38	9.95	0.36	0.35	5.88	0.07	207.06	267.33	0.46	3.82	99.76
LK12	95.00	7.57	0.04	0.09	10.57	0.09	106.74	109.78	0.65	1.69	100.76
LK13	5.00	33.58	1.76	4.85	11.88	0.11	90.67	80.77	0.82	1.88	7.65
LK14	49.38	25.35	1.60	5.09	14.85	0.07	104.45	67.54	0.75	1.75	8.12
LK15	41.88	21.57	1.62	4.88	10.94	0.09	106.42	78.09	0.45	1.93	9.05
Correlation of nutrients with DS		-0.948**	-0.826**	-0.916**	-0.015	-0.112	0.027	0.087	-0.132	0.167	0.773**

DS: Disease severity; LK1- LK2: Location in Andomesinggo village, Besulutu district, Konawe regency; LK6- LK8: Apeea village, Kecamatan Abuki district, Konawe regency; LK9- LK12: Pudambu village, Angata district, South Konawe regency; LK-13- LK15: Benua village, Benua district, South Konawe regency; **Correlation is significant at the 0.01.

Table 2: Cross-coefficient values of direct and indirect effects of leaf nutrient elements on the severity of VSD disease in cocoa.

Variable	Direct effect	Indirect effect through variable									
		N	P	K	Ca	Mg	Fe	Mn	B	Cu	Zn
N	-0.793	-	-0.332	0.130	0.048	0.004	0.008	-0.001	-0.018	-0.028	0.034
P	-0.392	-0.673	-	0.119	0.062	0.002	0.015	-0.034	0.035	0.006	0.033
K	0.147	-0.699	-0.318	-	0.026	-0.018	-0.011	-0.023	-0.008	-0.046	0.035
Ca	0.291	-0.130	-0.083	0.013	-	0.007	0.068	-0.038	-0.012	-0.151	0.019
Mg	-0.075	0.044	0.012	0.036	-0.027	-	-0.046	-0.055	0.001	0.002	-0.001
Fe	-0.119	0.056	0.048	0.014	-0.166	-0.029	-	0.029	0.036	0.178	-0.019
Mn	0.138	0.005	0.097	-0.024	-0.080	0.030	-0.025	-	-0.063	0.012	-0.004
B	-0.140	-0.104	0.099	0.008	0.024	0.000	0.031	0.062	-	-0.123	0.011
Cu	0.302	0.073	-0.008	-0.022	-0.146	0.000	-0.070	0.006	0.057	-	-0.024
Zn	-0.045	0.612	0.292	-0.115	-0.127	-0.002	-0.052	0.012	0.034	0.162	-

and Zn nutrients is capable of reducing disease severity. A deficiency in this micronutrient can make plants susceptible to pathogen attacks. The contribution of Cu to the increase in VSD can be mitigated by optimizing its indirect effects through N, P, Mn and Cu nutrients. As a result, the overall influence of Cu can reduce the severity of VSD. The direct impact of Cu on increasing disease severity can be minimized by optimizing its indirect effects through P, K, Ca, Fe and Zn nutrients, thereby reducing its overall influence.

The role of leaf mineral nutrition in reducing vsd disease severity

The leaf mineral nutrition variables in cocoa that had a significant direct negative impact on VSD disease severity were N and P, which was consistent with their correlation coefficients. In contrast, Mg, Fe, B and Zn had small direct negative effects (Table 2). These findings indicate that increasing the values of N, P, Mg, Fe, B and Zn directly reduces the severity of the VSD. The substantial direct effects of N and P in reducing VSD disease severity are strongly supported by the optimal status of N and P in cocoa leaf tissues, which effectively supports plant physiological processes.

The role of N in reducing the severity of VSD increases through its indirect influence on P, Mn, B and Cu. Nitrogen plays a crucial role in amino acid synthesis, photosynthesis, respiration and tricarboxylic acid cycle. N nutrients can enhance plant resistance through amino acid metabolism and hormone production to influence defense-related gene expression. However, excess N nutrition can also make plants susceptible to pathogen infections through the production of antimicrobial phytoalexins and physical defense mechanisms (Sun *et al*., 2020).

The role of P in reducing disease severity increases through its indirect influence via interactions with N and Mn. This nutrient functions to provide chemical energy storage and transfer as ADP and ATP, acts as a component and activator of enzymes and serves as a major component of DNA and RNA (Tripathi *et al*., 2022). P nutrient also plays an important role in reducing disease severity in several plants by activating defense responses by producing extracellular ATP (Jewell *et al*., 2019).

Magnesium, boron and zinc directly have a small influence on reducing VSD, corresponding to their generally suboptimal nutrient status. The indirect interactions of Mg with Ca, Fe, Mn and Zn further enhanced their role in reducing the VSD. Magnesium can enhance plant tissue resistance to cell degradation by the pectolytic enzymes produced by pathogens (Huber and Jones, 2013). The indirect influence of nutrients through N and Zn can reduce the severity of the VSD. Zinc can reduce disease severity indirectly through its interactions with K, Ca, Mg and Fe. However, Zn can indirectly increase disease severity through its interactions with N and P. Zn plays a role in influencing plant responses to pathogens, particularly in activating metalloenzymes and its application can reduce disease symptoms (Machado *et al*., 2018).

CONCLUSION

Based on the results, the following conclusions can be drawn:

1. VSD is related to the nutrient content in cocoa, where high disease severity is found in cocoa with low levels of N, P and K nutrients.
2. Mineral nutrients in cocoa have a direct impact on increasing the severity of VSD, with K, Ca, Mn and Cu nutrients playing a significant role, while N, P, Mg, Fe, B and Zn nutrients have a direct impact on reducing the severity of VSD.
3. The N and P nutrients have a significant direct influence, whereas K nutrients indirectly through N and P nutrients also play a significant role in reducing the severity of VSD. Efforts to reduce the severity of VSD in cocoa plants can be achieved through the enhancement of appropriate and complete nutrition, particularly N, P and K nutrients and few other nutrients like Mg, Fe, B and Zn.

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