

Foundation and validation of diagnosis and recommendation of integrated system norms for evaluating nutrient status of pineapple plants (*Ananas comosus* L.)

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Abstract

This study aimed to (i) establish the diagnosis and recommendation of integrated system (DRIS) norms for the evaluation of nutrient status in pineapple plants and (ii) validate the reliability of DRIS norms. Eighty-six leaf samples at +1 (E leaf) and +3 (D leaf) positions were collected from 86 pineapple fields cultivated in acid sulfate soils at the leaf development stage. Results indicated that DRIS norms established for pineapple included 22 and 30 pairs of nutrient ratios at E and D leaf positions, respectively. The validation for the reliability showed that founded DRIS norms were reliable and suitable for assessing the nutrients status of pineapple. Based on the fertilizer omission plot technique, DRIS indices of N, Ca, and Mg in the treatments without N, Ca, or Mg fertilizer, respectively, were less excessive than those in the treatment with full NPKCaMg fertilization at both leaf positions, meaning that the DRIS norm is reliable. Both leaf positions can be used to diagnose N, K, Ca, and Mg nutrients status.

Keywords: acid sulfate soils; DRIS; leaf nutrition; foliar diagnosis; nutritional diagnosis; pineapple.

Introduction

Pineapple (*Ananas comosus* L.) is one of the most important tropical fruits of the globe (Baruwa, 2013) for its high content of minerals and vitamins (Hossain et al., 2015). In Vietnam, the total area for pineapple cultivation is approximately 36,658 ha, producing 617,944 t of pineapple (FAOSTAT, 2022). The Queen pineapple has been cultivated for a long time and is intensively grown in the provinces of the Mekong Delta, including Tien Giang, Kien Giang, Long An, and Hau Giang, accounting for 70% of national pineapple production. Huu et al. (2022), studied nutrients of pineapple fruit, by which the biomass, phosphorus content, water content, titratable acidity, vitamin C, Brix degree and pH was roughly 2,671 kg ha⁻¹, 0.764%, 173.1 mL fruit⁻¹, 0.344 g_{citric acid} 100 g_{flesh weight}⁻¹, 16.5 mg 100 g⁻¹, 8.96, and 3.72. Pineapple is able to grow strongly on acid sulphate soils (Ganeshamurthy et al., 2016), which exists in Hau Giang province, Vietnam. These pineapple productions were harmless and safe for human consumption and health (Le et al., 2022). In this province, pineapple is the second most grown plant after rice. Historically, cultivation techniques and fertilizer application were just based on farmers' experience, and the balance in supplying nutrients has not been properly maintained. This caused adverse effects in cultivation though no official publications considered the prevalence of nutritional deficiencies. Meanwhile, Amorim et al. (2013) stated that balanced nutrient supplementation was the key factor affecting the yield and quality of pineapple. However, the demand for nutrients, including N, P, K, Ca, and Mg, of the pineapple differs according to the

stages of its life cycle (Montoya et al., 2018). Furthermore, nutrient values at different leaf positions vary as well. Therefore, the selection of leaf position is crucial to evaluate the nutrient status of pineapple, and the diagnosis and recommendation integrated system (DRIS) is recognized as a potential solution. To be more specific, Beaufils (1973) has founded DRIS norms to assess the nutrient status of pineapple via leaf nutrition content. The DRIS index is able to estimate the nutrient balance in plants and to relatively categorize nutrient levels. Specifically, a negative DRIS index highlights insufficient nutrition, a positive DRIS index represents excess nutrition, and a DRIS index at zero means balance. The DRIS technique has been applied for assessing nutritional imbalances in crops such as bananas and acai palm (Neto et al., 2022; Ribeiro et al., 2020). Up to now, there have been 5 DRIS norms for pineapple created in the world (Sema et al., 2010; Teixeira et al., 2009; Agbangba et al., 2011; Angeles et al., 1990; Montoya et al., 2018). Nevertheless, each site has a specific climate and soil properties, so DRIS norms from another site may have lower reliability. Until now, the nutrition diagnosis results have relied on the DRIS norms of D leaf and different sampling times. Although the method has been widely used in different fruit crops, DRIS for the Queen pineapple (Cau Duc pineapple) had just been developed in preflowering stage in Mekong Delta, Vietnam (Quoc et al., 2022). However, the leaf development stage is very important to estimate the nutrition status due to its role in formation of fruit. Thus, the objectives of the study were to (i) find DRIS norms to assess

the status of macro- and micronutrients in pineapple at the leaf development stage and (ii) confirm the founded DRIS norms based on a nutrient omission plot of each N, P, K, Ca, and Mg fertilizer for pineapple.

Results

Norms foundation of the diagnosis and recommendation integrated system for evaluating the nutrient status of pineapple

Norms foundation of DRIS from E leaf position

The pineapple yield had significant differences at 1% between the high and low yield groups, whose values were 17.1 and 12.7 t ha⁻¹, respectively (Supplementary Table 1). Moreover, the average yield was 14.9 t ha⁻¹. Consequently, the pineapple fields which had a yield higher than 14.9 t ha⁻¹ were classified as a high yield group (29 fields) and the others whose yields were lower than 14.9 t ha⁻¹ made up the low yield group (57 fields), accounting for 33.7 and 66.3%, respectively. In addition, the concentration of nutrients, including N, P, K, Ca, Mg, and Mn, varied significantly at 1, 5, or 10%, while the concentrations of Na, Cu, and Zn were statistically similar in both yield groups (Supplementary Table 1).

The variance ratio between the low and the high yield groups (S^2/S^2_h) was significantly high, which meant that the nutrition pairs potentially reached a high yield. This emphasized that the DRIS index selected for the nutrition pairs had better S^2/S^2_h value. Thus, the selected nutrition pairs were 36 (Supplementary Table 2). Additionally, in the case that the difference between the yield groups was insignificant and was not caused by nutrients difference, the founded DRIS norms were not reliable enough for diagnosis. Thus, 22 nutrition pairs were chosen to be DRIS norms for pineapple cultivated in acid sulfate soil at E leaf position during the leaf development stage (Supplementary Table 2).

Norms foundation of DRIS from D leaf position

Supplementary Table 3 showed that the concentrations of nutrients, including N, P, K, Ca, and Mg, in the high yield group were remarkably different at 1% compared to those in the low yield one. Notwithstanding, Na concentration in the low yield group was higher than that in the high yield one, 0.21 ppm compared to 0.09 ppm, respectively. Moreover, the content of Cu, Zn, and Mn pinpointed insignificant differences between the low and high yield groups.

In the same line as the DRIS index, the variance ratio was selected among nutrition pairs in both yield groups. According to the S^2/S^2_h ratio, there were 36 nutrition pairs selected. Among them, the 30 pairs chosen based on DRIS norms at the D leaf position during the leaf development stage are illustrated in Supplementary Table 4. This implies that the selected pairs differ from each other statistically at 1% in mean values and variances between the low and high yield groups.

Validation of founded norms based on nutrient omission trial

Validation of the founded DRIS norms based on E leaf position

The results in Supplementary Table 5 illustrated that all of the treatments had excessive N concentrations. Yet, the DRIS index obtained the least excessive N in the treatment without fertilization and the treatment with the omission of N fertilization, whose values were 141.6 and 142.1, respectively. The DRIS index in farmers' fertilizer practice

(FFP) treatment was 161.6, higher than that in the treatment fertilized with all nutrients (158.6) and the one without N fertilization (142.1). This revealed that the N dose farmers supplied to the pineapple was higher than its demand.

For P, the DRIS index in the NPKCaMg treatment gave the most serious shortage of P (-379.3), and, in the treatments without N, K, and Mg, the pineapple was more deficient of P than that in the treatment without P, sequentially -249.6, -229.1, and -240.0. Accordingly, there was no exact evaluation of P status in the E leaf position in this study. Similarly, the DRIS index in the treatment without K was -30.4, significantly lower than the treatments with omissions of N, P, and Mg and the NPKCaMg treatment, whose results were -8.1, -9.6, -3.4, and -21.2, respectively (Supplementary Table 5).

The DRIS index in Supplementary Table 5 exposed that, in the NPKCaMg treatment, the amount of excessive Ca peaked at 1,036.0. Additionally, in the treatment without Ca, the overabundant Ca value was much lower than that in the FFP treatment (354.0). Hence, more Ca was added to the soil by local farmers than was necessary for the pineapple crop. Furthermore, the Mg value in the treatment without Mg supplementation amounted to the lowest excessive value, 187.1, compared to that of the other treatments. Thus, according to Supplementary Table 5, a conclusion could be made that the N, Mg, and Ca used by local farmers were too much for the pineapple demand, but the K content did not meet its need.

Validation of the founded DRIS norms based on D leaf position

The DRIS index demonstrated the lowest N abundance in the treatment without N fertilization (89.8) and the highest in the FFP treatment (113.3) (Supplementary Table 6).

The DRIS indices of P in the treatment without fertilization and the one without Ca fertilizer were less deficient than those of the other treatments and were valued at -103.6 and -115.4, respectively. Additionally, the DRIS index of P in the treatment not fertilized with P was -124.0, remarkably higher than those of the NPKCaMg, PKCaMg, and NPCaMg treatments, whose results were -132.0, -127.8, and -124.3, respectively (Supplementary Table 6).

Sharing the same trend, for K, the DRIS index in the treatment without K was higher than those of the NPKCaMg, PKCaMg, NPKMg, and NPKCa treatments, with corresponding numbers of 334.7, 320.3, 272.7, and 339.3. On the other hand, the Ca content in the FFP treatment was 192.1, less excessive than the treatment without Ca (245.8). Further, the DRIS index of Mg in the treatment without Mg was 95.0, higher than those of the control, NPKCaMg, and no P fertilization treatments (84.9, 90.9, and 88.0, respectively) and lower than those of the other treatments (Supplementary Table 3).

To sum up, the DRIS norms built for pineapple at both E and D leaf positions during their development stage were highly reliable for status of nutrients, including N, Ca, and Mg. Nevertheless, the system had low reliability for P at both leaf positions and K at the D leaf position, leading to the fact that the determination for abundance and deficiency of P and K was not revealed in this study.

Discussion

The soil analysis revealed that soil chemical properties could affect pineapple yield. Among them, pH_{KCl} , $\text{pH}_{\text{H}_2\text{O}}$, Fe^{2+} , Al^{3+} , soluble P, and insoluble P compounds (Al-P, Fe-P, and Ca-P)

were believed to be at harmful or limiting levels for plant growth (Supplementary Table 7). This was in accordance with the cultivated soil status for pineapple; the toxicity of Al^{3+} and Fe^{2+} in the soil was at a high level according to the evaluation of Taylor et al. (1966). In the same line, under low pH conditions in acidic soil, the solubility of soil metals is affected, creating toxicity of Fe^{2+} and Al^{3+} due to a high dissolved concentration (Rieuwerts, 2007). Too, the toxicants fixed with P form insoluble P compounds, negatively influencing plant yield (Margenot et al., 2017) and producing low available P content in the soil. This is because P was fixed by metal ions, which prevented plants from P uptake and ultimately resulted in lowering the efficacy of P use (Malhotra et al., 2018).

Apart from toxicity, Fe^{2+} and Al^{3+} also compete with other cations on the root absorbing surface to fix P in insoluble forms or Fe_2O_3 forms attaching to the root. Hence, the uptake of water and nutrients is limited (Acevedo-Gómez et al., 2020). In soil, iron and aluminum ions can compete in absorbing positions in soil particles with other ions, encompassing NH_4^+ , Ca^{2+} , Mg^{2+} , and K^+ , generating lower cation exchange capacity and a lower concentration of those cations in the soil. This is consistent with the soil analysis result of Horneck et al. (2011), which reports that Ca^{2+} and Mg^{2+} exchange capacity in low and intermediate levels, respectively, when there is a large amount of Fe and Al found in the soil. The result accorded with the report of Bravo et al. (2017) which states that, for low pH (< 5.0), the usable values of N, P, K, Ca, Mg, Cu, and Zn go down and nutrient deficiency appears, at which point there is a rise in the number of Fe and Al contents. All of the factors above are regarded as obstacles to pineapple cultivation. This provoked lower pineapple yields compared to those of other sites in the world (Agbangba et al., 2011; Angeles et al., 1990; Montoya et al., 2018; Sema et al., 2010; Teixeira et al., 2009).

The obstacles of soil in cultivating pineapple fire up changes in the nutrition status of pineapples via their nutrient uptake. Therefore, DRIS norms were applied in order to assess nutrition status based on nutrients in pineapple leaves, instead of the nutrient concentrations in soil. The DRIS norms performed in this study were a useful tool for assessing the stages of excess, deficiency, and balance in order to supply the suitable macro- and micronutrients for pineapple (Tables 1 and 2). Meanwhile, leaves develop in all stages of the pineapple cycle and the D leaf shows a clear growth index in pineapple nutrition (Paull and Duarte, 2011), as well as the yield at harvest (Mahmud et al., 2018). Furthermore, sampling leaves at a suitable position and time is the key step to building a suitable DRIS. Nutrition status in plants is exposed via the concentration of nutrients in leaves and changes according to leaf age and growth stage, especially in the reproductive and vegetative stages (Serra et al., 2013). Leaf positions E and D are two leaf types which are fully developed and sensitive to the changes in the nutrient concentrations in soil. This is why E and D leaf positions of the leaf development stage were chosen to create DRIS norms set for pineapple cultivated in acid sulfate soil.

Collectively, the nutrient content in the leaves was equivalent to that of previous studies (Supplementary Table 1). Specifically, the N and K concentrations at E leaf position are consistent with the result of a study done by Angeles et al. (1990). After all, the P concentration ranged from 0.48 to 1.00%, noticeably higher than that in the studies by of Sema

et al. (2010) and Angeles et al. (1990), which fluctuated from 0.08 to 0.23%. This can be explained by the fact that the values were dependent on the soil, climate condition, and cultivation techniques of each region, as well as the dose and type of P fertilizer used by farmers (Malézieux et al., 2003). Together, the result relating to Ca, Mg, and Cu concentrations was consistent with a previous study by Sema et al. (2010). The amount of nutrients in D leaf position (Supplementary Table 3) concurred with the study of Sema et al. (2010), in which the optimum nutrition in leaves to build the DRIS norms set consisted of 1.21 – 1.85% N, 0.13 – 0.18% P, 1.19 – 1.62% K, 0.27 – 0.35% Ca, 0.43 – 0.56% Mg, 41.5 – 58.3 ppm Mn, 7.4 – 10.2 ppm Cu, and 12.2 – 15.8 ppm Zn. Angeles et al. (1990), reported that the contents of P and K are higher than those in other studies but still in the range of other important norms developed previously. In addition, Silva et al. (2009) have reported that the nutrient demands of pineapple were much higher than those of other plants and relied on pineapple cultivars, fruit weight, planted location, plant density, and the cultivation system.

Additionally, Letsch and Sumner (1984) have claimed that, in order to assure the accuracy of the remarkable differences between the two yield groups, the high yield one should account for at least 10% of the database and the percentage of the high yield group attained 33.7% (Supplementary Table 1). In addition, the coefficient of variation was quite low (<26%), suitable for diagnosing the nutrition status of nutrients (Walworth and Sumner, 1987), because a low CV value means high reliability of DRIS norms. Nevertheless, the CV was high in the study (Supplementary Tables 2 and 4), because there were differences in production among farming households in each group (Supplementary Table 1). For founded DRIS norms, a greater nutritional pair's variance ratio S^2_i/S^2_h is selected because the DRIS index makes pairs of nutrition ratios between the low and the high yield groups (Payne et al., 1990; Serra et al., 2013). Moreover, the statistical differences between the two groups unveil high reliability in constructing DRIS norms (Abebe et al., 2018). All of the selected nutrition pairs for DRIS norms had significantly different mean values. This proved the reliability of the DRIS norms found in the study (Supplementary Tables 2 and 4; Tables 1 and 2). This was also affirmed in the study done by Payne et al. (1990). The DRIS norms consisted of means, coefficient of variation, variance, and variance ratio between the low and the high yield groups (Supplementary Tables 2 and 4).

The nutrition pairs chosen, comprising Cu, Zn, and Mn, had a variance ratio between the low and high yield groups higher than 2. This result agreed with a study by Payne et al. (1990) on bahiagrass, which has twenty-three pairs of nutrient ratios with $S^2_i/S^2_h > 2$ out of thirty-six nutrition ratio pairs selected following DRIS norms and 17 pairs of nutrient ratios of micronutrients (Cu, Mn, and Zn). This stated the importance of DRIS norms to micronutrients due to high variance, evoking difficulty in obtaining the proper nutrition ratio for a high yield, and the accuracy of the micronutrient fertilizer demand for pineapple was determined by soil experiments. Bailey et al. (1997) conclude that nutrition pairs with high S^2_i/S^2_h and small CV express balance, which is extremely crucial in manufacturing. Thus, nutrition pairs with high S^2_i/S^2_h and small CV suggest little fluctuation in the high yield group.

Taken together, DRIS norms were founded for pineapple (Supplementary Table 2; Tables 1 and 2), and the sensitivity

Table 1. The selected DRIS norms, coefficient of variation (CV), variance (S^2) and the variance ratio (S^2_i/S^2_h) of nutrient ratios of the low and high yield groups of pineapple cultivated in acid sulfate soil in E leaf.

Nutrient	High yield group (n=29)			Low yield group (n=57)			S^2_i/S^2_h
	Mean	CV (%)	Variance (S^2_h)	Mean	CV (%)	Variance (S^2_i)	
N/P	2.06***	27.1	0.31	3.22	36.9	1.41	4.51***
N/K	0.78**	28.8	0.05	0.92	37.5	0.12	2.39**
Na/N	0.07***	67.7	0.002	0.09	37.6	0.001	0.44***
Cu/N	7.55***	38.3	8.35	9.66	47.2	20.80	2.49**
K/P	2.87***	42.4	1.47	3.79	43.3	2.69	1.83*
Ca/P	0.20***	24.0	0.002	0.33	49.2	0.026	11.1***
Mg/P	0.28***	26.6	0.005	0.47	40.9	0.037	6.72***
Cu/P	14.9***	40.7	36.9	29.4	55.8	270	7.32***
Zn/P	116.5***	46.1	2,890	239.5	54.7	17,167	5.94***
Mn/P	79.1***	43.9	1,203	217.4	52.0	12,795	10.6***
Cu/K	5.51***	34.8	3.67	8.55	58.1	24.7	6.71***
Zn/K	43.3***	44.4	368.6	72.6	65.6	2,266	6.15***
Mn/K	31.2***	60.5	357.7	61.3	46.8	823.3	2.30**
Cu/Ca	74.4***	35.2	685.1	108.0	62.8	4,600	6.72***
Zn/Ca	565.9***	36.8	43,489	939.3	84.6	631,487	14.5***
Mn/Ca	390.5***	37.7	21,678	828.1	80.5	444,711	20.5***
Cu/Mg	54.7**	38.1	433.3	69.7	56.9	1,573	3.63***
Zn/Mg	433.7**	48.8	44,786	581.2	61.2	126,622	2.83**
Mn/Mg	298.7***	47.6	20,249	482.6	40.0	37,354	1.84*
Mn/Na	661.2**	47.4	98,305	916.1	55.5	258,516	2.63**
Mn/Cu	5.98**	56.1	11.64	8.76	63.3	30.7	2.64**
Mn/Zn	0.75**	41.7	0.102	1.02	53.1	0.29	2.88**

Mean of nutrient ratios of low and high yield groups are significantly different at 1% (***) , 5% (**) and 10% (*) level of probability by T test; variances of nutrient ratios of low and high yield groups are significantly different at 1% (***) , 5% (**) and 10% (*) level of probability by F test.

Table 2. The selected DRIS norms, coefficient of variation (CV), variance (S^2) and the variance ratio (S^2_i/S^2_h) of nutrient ratios of the low and high yield groups of pineapple cultivated in acid sulfate soil in D leaf.

Nutrient	High yield group (n=29)			Low yield group (n=57)			S^2_i/S^2_h
	Mean	CV (%)	Variance (S^2_h)	Mean	CV (%)	Variance (S^2_i)	
N/P	2.49**	24.1	0.36	3.18	29.3	0.87	2.41**
N/K	0.71**	27.8	0.04	1.02	58.3	0.35	9.09***
N/Ca	10.3**	24.8	6.46	14.8	58.3	74.7	11.7***
Na/N	0.04**	37.0	0.000245	0.14	60.4	0.007	30.4***
Cu/N	6.95**	44.3	9.49	11.4	37.4	18.0	1.90*
Zn/N	58.7**	45.8	721.8	89.1	57.4	2,615	3.62***
Mn/N	38.2**	46.1	309.2	65.7	56.4	1,371	4.44***
P/K	0.30*	36.9	0.012	0.43	99.5	0.18	14.9***
Mg/P	0.35**	32.4	0.013	0.47	37.6	0.031	2.42**
Na/P	0.10**	39.1	0.002	0.44	61.3	0.072	44.1***
Cu/P	16.9**	42.3	50.9	34.7	38.0	173.8	3.42***
Zn/P	143.2**	48.3	4,790	265.7	52.4	19,382	4.05***
Mn/P	93.0**	48.3	2,018	199.4	59.5	14,064	6.97***
Ca/K	0.07*	28.4	0.000409	0.11	129.1	0.021	51.1***
Mg/K	0.10**	40.5	0.002	0.18	93.2	0.029	17.6***
Na/K	0.03**	34.8	0.000102	0.14	79.8	0.010	128.6***
Cu/K	4.88**	52.3	6.52	15.7	122.6	370.5	56.9***
Zn/K	41.0**	50.3	425.5	100.32	82.1	6,778	15.9***
Mn/K	26.2**	50.8	176.9	85.9	115.9	9,896	55.9***
Mg/Ca	1.46**	41.9	0.38	2.32	86.1	4.01	10.7***
Na/Ca	0.43**	39.3	0.028	2.18	86.6	3.55	124.7***
Cu/Ca	69.2**	45.1	9,74.6	176.1	79.5	19,606	20.1***
Zn/Ca	581.5**	43.4	63,642	1,465	99.1	2,107,023	33.1***
Mn/Ca	379.7**	47.3	32,259	1,075	98.9	1,129,934	35.0***
Na/Mg	0.32**	46.8	0.022	1.03	66.9	0.47	21.2***
Cu/Mg	52.1**	47.8	621.7	80.4	43.3	1,210	1.95*
Mn/Mg	291.1**	57.2	27,723	460.6	57.2	69,305	2.50**
Na/Cu	0.01**	53.1	0.000015	0.01	71.4	0.000099	6.47***
Na/Zn	0.002**	38.4	0.0000001	0.001	83.5	0.00003	29.3***
Na/Mn	0.002**	47.4	0.0000004	0.001	65.6	0.000029	8.02***

Mean of nutrient ratios of low and high yield groups are significantly different at 1% (***) , 5% (**) and 10% (*) level of probability by T test; variances of nutrient ratios of low and high yield groups are significantly different at 1% (***) , 5% (**) and 10% (*) level of probability by F test.

of the system was evaluated based on the comparison of nutrient concentrations in leaves (at the same sampling time and position) between the treatment fertilized with N, P, K, Ca, and Mg and the ones with omitted fertilization according to each nutrient (Supplementary Tables 5 and 6). The evaluation result of the formed DRIS norms manifested that, at the E leaf position, the DRIS index of N in the treatment fertilized as FFP was more excessive than the treatment without N fertilizer. Furthermore, the DRIS index of Ca in the treatment without Ca was less excessive than the FFP treatment, and the Mg content in the treatment without Mg got the lowest abundant amount. The DRIS index of K in the treatment without K fertilizer was more deficient than that of the treatments without N, P, and Mg and the fully fertilized treatment (Supplementary Table 5). This marked that local farmers overused N, Mg, and Ca fertilizers and did not use adequate K fertilizer, in comparison to the pineapple requirement. As reported by Bailey et al. (1997), excessive fertilization of N and K causes a shortage of Mg. Additionally, at D leaf position, in the case in which N fertilizer was not applied, the DRIS index exhibited the lowest value, 89.8, and the most abundant N content belonged to the FFP treatment, which was 113.3 (Supplementary Table 6). In the case in which P fertilizer was not used, Ca and Mg uptake was reduced, inducing a lower number in the index of Mg than that in the treatment without Mg fertilization. Too, the overuse of N fertilizer decreases pineapple fruits' quality, i.e., an increase in the fruit pulp's pH and lower acid content (Bonomo et al., 2020). According to the report of Rios et al. (2018), using K up to 410.4 kg ha⁻¹ increases the carbohydrate content and the fruit's weight, and K deficiency induces low quality of pineapple fruits (Cunha et al., 2021). For Victoria pineapple to gain a yield of 72 t ha⁻¹, the plants need to uptake 452 kg N ha⁻¹ (Pegoraro et al., 2014). The determination of the system's sensitivity is essential because the nutrition ratio of the system is equivalent to the ratio on the field; thus, that field will gain a high yield (Reis and Monnetrart, 2003).

Materials and methods

Foundation of DRIS

Soil sampling

There were 86 soil samples collected at depths of 0 – 20 cm from 86 pineapple fields in Long My district and Vi Thanh city, Hau Giang province, from March 2019 to September 2020, in order to analyze the chemical properties of acid sulfate soil (Umbric Gleysol [Epi ortho thionic]) as the classification of FAO (2014) following the guideline of Tan (2005). Their characteristics were recorded in Supplementary Table 7.

Soil preparation

The soil was left to dry naturally; then, it was smashed and ground via sieves of 0.5 and 2.0 mm in order to be analyzed.

Soil analysis

All of the methods used to analyze the soil in this study were summarized by Sparks et al. (1996).

Leaf sampling and preparation

In order to found DRIS norms, 86 healthy pineapple leaf samples were collected at +1 (E leaf) and +3 (D leaf)

positions (Supplementary Figure 1) during their development stage (7 months after cultivation) in Long My district and Vi Thanh city, Hau Giang province, Vietnam. Each sample consisted of 20 uninfected leaves. After the impurities were eliminated, the leaves were dried at 70 °C for 5 days until being completely dry, and then they were ground for nutrition analysis. This protocol was performed under the guidelines of McCray et al. (2015).

Leaf analysis

The concentrations of leaf samples were detected following the method of Houba et al. (1997).

Pineapple fruit yield

The weights of pineapple fruits of 5 m² at harvesting were recorded for each field that collected foliar samples. Then, the yield was converted into t ha⁻¹. Based on the mean productivity value, the farming households were divided into high and low yield groups.

DRIS norms foundation

The DRIS norms were founded based on the studies by Beauflis (1973) and Walworth et al. (1986) and arranged as a result of the following steps:

Step 1. Calculate all pairs of nutrients (Supplementary Tables 2 and 4).

Step 2. Calculate the means, coefficient of variation, variance, and variance ratios between the high and low yield groups.

Step 3. The mean yield and nutrition concentrations of the high yield group were compared with those of the low yield one. The differences between nutrition pairs were checked. Then, the pairs with no significant difference were deleted, and from that DRIS norms set was established for pineapple. Furthermore, two criteria were suggested for choosing pairs for DRIS: (i) the average concentration of nutritional ratio between high and low yield groups differed from each other significantly at 5% and (ii) the nutrition pairs had a variance ratio between the low and high yield groups (S^2_l/S^2_h) higher than that of other pairs.

Validation of the founded DRIS norms

Field experimental design

The experiment was conducted in a completely randomized block design with 8 treatments and 4 replicates each as follows: (i) NF, no fertilization; (ii) NPKCaMg, plot fully fertilized with N, P, K, Ca, and Mg fertilizers; (iii) PKCaMg, N omission plot, which was fertilized with P, K, Ca, and Mg; (iv) NKCaMg, P omission plot, which was fertilized with N, K, Ca, and Mg; (v) NPCaMg, K omission plot, which was fertilized with N, P, Ca, and Mg; (vi) NPKMg, Ca omission plot, which was fertilized with N, P, K, and Mg; (vii) NPKCa, Mg omission plot, which was fertilized with N, P, K, and Ca; and (viii) FFP, farmers' fertilizer practice. The recommended fertilizer formula for pineapple in the Mekong Delta consisted of 10 g N – 9 g P₂O₅ – 8 g K₂O – 40 g CaO – 20 g Mg plant⁻¹.

Sampling leaves for evaluating the founded norms

Twenty healthy leaves per sample at each position of E and D leaf during the leaf development stage in every treatment were sampled. The leaf samples were prepared and analyzed as described in Section 2.1.

The method of evaluating the sensitivity of founded DRIS norms

Step 1. Calculate the nutrition ratio for every pair of nutrients following the standard of Elwali and Gashcho (1984) DRIS, including N/P, P/N, N/Ca, Ca/N, N/K, K/N, K/Ca, Ca/K, N/Mg, Mg/N, Ca/Mg, Mg/Ca, P/K, K/P, P/Mg, Mg/P, K/Mg, Mg/K, Ca/P, and P/Ca.

Step 2. Calculate the DRIS index based on Walworth and Sumner's (1987) formula:

$$\text{Index A} = \frac{[f(A/B) + f(A/C) + f(A/D) \dots + f(A/N)]}{Z}$$

$$\text{Index B} = \frac{[-f(A/B) + f(B/C) + f(B/D) \dots + f(B/N)]}{Z}$$

$$\text{Index N} = \frac{[-f(A/N) - f(B/N) - f(C/N) \dots - f(M/N)]}{Z}$$

where A/B is not lower than a/b, $f(A/B) = [(A/B)/(a/b) - 1] (1,000/CV)$, and

A/B is lower than a/b, $f(A/B) = [1 - (a/b)/(A/B)] (1,000/CV)$.

A/B is the nutrition ratio of a pineapple for diagnosis, a/b is the ratio in founded DRIS norms, CV is the coefficient of variation, and Z is the number of functions calculated in the overall nutrients.

Evaluating DRIS index

The DRIS index with a negative value represents insufficient nutrition, that with a positive value stands for excessive nutrition, and that with a zero value symbolizes balanced nutrition.

Statistical analysis

The data were processed by Microsoft Excel version 2013. T-test was used to compare the means of pineapple yield and nutrient content of 2 yield groups, and F-test was applied to compare variances among nutrition pairs of N, P, K, Ca, Mg, Na, Cu, Zn, and Mn.

Conclusions

The DRIS norms were founded on the nutrient concentrations in pineapple leaves at E and D positions during the leaf development stage in order to perform the nutrition diagnosis for pineapple. The DRIS norms during the leaf development phase were derived from 22 nutrition pairs at the E leaf position and 30 ones at the D leaf position. Significant differences were documented in the mean content and variance ratios between the low and high yield groups. Based on omitted fertilization according to nutrients, including N, P, K, Ca, and Mg, the founded DRIS norms confirmed high reliability. During the leaf development period, at E and D leaf positions, the DRIS indices of N, Ca, and Mg in the treatments without N, Ca, and Mg, respectively, were less excessive than that of the fully fertilized treatment and the FFP treatment. Moreover, the K content at the E leaf position in the treatment without K was more deficient than that in the NPKCaMg treatment. Nonetheless, the founded DRIS norms did not estimate the abundant and deficient status of K at the D leaf position and P at both E and D leaf positions.

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