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ORIGINAL ARTICLE

Effect of calcium and silicon fertilization after flowering on pineapple mineral status and flesh translucency

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Abstract Proper calcium fertilization can reduce flesh translucency, while silicon fertilization can enhance fruit quality and mineral content, mitigating physiological disorders. Therefore, this study investigated the effect of calcium and silicon fertilization after flowering on pineapple mineral status and flesh translucency. Treatments were, A (control: Without Ca and Si), B (Ca from ten weeks before harvest until harvest), C (Ca from six weeks before harvest until harvest), D (Si from ten weeks before harvest until harvest), E (Si from six weeks before harvest until harvest), F (Ca + Si from ten weeks before harvest until harvest), and G (Ca + Si from six weeks before harvest until harvest). Flesh translucency, fruit (Ca, K, Mg, B, and Si), and crown (Ca and Si) mineral content were determined. In the first trial, treatment E had the best performance, essentially it because increased the fruit mineral content (Ca:1843, K:16,346, and Si: 2140 mg kg^{-1}, respectively), and produced the lowest translucency incidence (5%). In the second trial, the best performance was observed in treatment B, having the lowest translucency incidence (5%), despite not increasing the fruit mineral content. A cell wall analysis proved that the high calcium and silicon ions assimilation was essential to reduce the translucency incidence (Ca:22.60 and Si:3.29 weight%, respectively). In conclusion, calcium and silicon fertilization after flowering can reduce translucency, impacting the fruit mineral status. More experiments should be done on calcium and silicon influences on fruit and crown physiology and their relation with translucency.

Keywords Abiotic · Cell wall · Incidence · MD2 · Stress · Waterlogging

Introduction

Pineapple (*Ananas comosus* L. Merr.) is economically a valuable crop in several countries (Cano-reinoso et al., 2021a, 2021b; Hossain, 2016). Currently the industry frequently requires low acid hybrids. However, a crucial problem of these hybrids is their susceptibility to excessively low acidity, promoting an undesirable increase in the total soluble solids, and physiological disorders like translucency (Cano-reinoso et al., 2021a, 2021b; Murai et al., 2021).

Translucency is a physiological complication affecting the pineapple flesh characterized by water soaking symptoms, including low porosity (Murai et al., 2021; Paull & Chen, 2018). Despite its unknown cause, translucency has been investigated since time ago (Paull & Chen, 2015, 2018). Chen and Paull (2017) and Murai et al. (2021) suggested that temperature, sugar accumulation and calcium level were essential factors related to this physiological disorder.

Calcium is an essential mineral that maintains the cell wall's constitution and turgor (De Freitas & Resender Nassur, 2017; Hocking et al., 2016). Furthermore, calcium signalling into several proteins stabilize the cell wall's integrity and regulate the solute interchange between the cell's interior and exterior (De Freitas & Resender Nassur,

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2017; Hocking et al., 2016). Calcium ions (Ca^{+2}) are mobile in the plant's xylem but not in the phloem (Hocking et al., 2016). Therefore, as the fruit is the main sink for the plant, the calcium level decrease in concomitance with its ripening; which is a typical pattern in pineapple (Paull & Chen, 2015, 2018). Vásquez-Jiménez and Bartholomew (2018) explained that pineapple is a crop that after flowering does not assimilate substantial quantities of minerals through root absorption and translocation. Therefore, foliar applications of necessary elements like calcium become a solution to supply the fruit with this element.

Another mineral that has been studied as a physiological signalling regulator in plants is silicon. Studies on silicon have exposed that it influences the cell and membrane properties (Liang et al., 2015). However, despite this fact, it is still not clear its mechanism of interaction inside the plant physiology (Liang et al., 2015). On the other hand, foliar use of silicon has emerged as a complementary application (Laane, 2018; Liang et al., 2015). Studies have proved that the quality improvement on crops is similar or better than soil silicate fertilization in several cases (Laane, 2018). Foliar fertilization with stabilized silicic acid improved root and plant growth, yield and quality in any soil type, including monocots and dicots plants (Artyszak, 2018; Laane, 2018). Furthermore, these sprays were remarkably efficient against diverse biotic and abiotic stresses (Artyszak, 2018; Laane, 2018).

Fewer than ten papers contained information about this mineral in pineapple have been found, with mostly its presence in plant ash (Vásquez-Jiménez & Bartholomew, 2018). Nevertheless, because of the demonstrated beneficial effect of silicon on managing plant stresses and enhancing several crops' quality, this mineral turns out to be a possible solution to reduce and control the pineapple translucency occurrence.

Therefore, as foliar calcium formulation, doses, and application time are important to control translucency, due to the reduction of mineral assimilation after flowering. Also, because of the positive effects that silicon could provide, not only controlling translucency, also improving mineral uptake in pineapple after flower induction; this study aims to evaluate the effect of calcium and silicon fertilization after flowering on pineapple mineral status and flesh translucency, focused on a low acid hybrid and the fruit mineral impact.

Material and methods

Materials and experiment design

The research was conducted in 2020 in pineapple fields of Lampung, Sumatra Island of Indonesia. MD2 Pineapple low acid hybrid was used. The harvesting of the fruit was carried out between 144 to 147 days after flowering, when it is considered MD2 pineapple exhibit the appropriate physical and chemical characteristics to be consumed (Bin Thalip et al., 2015; Ding & Syazwani, 2016).

The research was layout between February to April and replicated from May to July of 2020. In the two trials employed, the flower induction and fruit development happened through the rainy season. Table 1 describes the physical and mineral characteristics of the soil where the research was implemented.

The soil had previously been fertilized with 200 kg ha^{-1} Di-ammonium Phosphate, 1000 kg ha^{-1} K_2SO_4, and 200 kg ha^{-1} Kieserite crystal at the moment of row preparations; after that, foliar applications of 700 kg ha^{-1} Urea, 700 kg ha^{-1} $(NH_4)SO_4$, 1000 kg ha^{-1} K_2SO_4, 170 kg ha^{-1} $MgSO_4$, 60 kg ha^{-1} $FeSO_4$, and 60 kg ha^{-1} $ZnSO_4$, were carried out from three months after plating in intervals of 30 days. Finally, following the flower induction, liquid Ethepon and Borax were sprayed in doses of 2.5 L ha^{-1} and 30 kg ha^{-1}, respectively. A weather station (LSI Lastem, equipped with a CR6 datalogger of Cambell scientific, Italy) calculated an average of 71.66% of relative humidity (RH), 23.45 °C of ambient temperature, and 16.83 w m^{-2} of solar radiation for the period of the first trial; while for the trial two, these variables were in average 89.34% RH, 26.8 °C, and 9.18 w m^{-2}, respectively. The monthly data of the rainfall through the experiment time for both trials are presented in Table 2.

A randomized complete block design was implemented. Seven treatments with four replications were arranged with 44 fruits per replication. There were also seven rows in each block with a width and length of 0.4 and 3.75 m. Pineapple plants were set in two lines of 22 plants inside

Table 1 Physical and mineral properties of the soil in the experiment

Texture	1st Trial	2nd Trial
Clay (%)	8.00	21.92
Loam (%)	39.56	10.72
Sand (%)	52.44	67.36
Chemical properties	**1st Trial**	**2nd Trial**
pH (H_20)	7.69	5.33
C (%)	3.64	0.90
N (mg kg^{-1})	950.00	830.00
P (mg kg^{-1})	1.32	11.50
K (mg kg^{-1})	6.68	156.00
Ca (mg kg^{-1})	66.30	212.00
Mg (mg kg^{-1})	104.00	100.80
Na (mg kg^{-1})	10.80	6.90

Table 2 Monthly data of the rainfall through the experiment

Month (mm)	1st Trial	Month (mm)	2nd Trial
February	457.7	May	106.4
March	282.1	June	199.9
April	262.9	July	95
Cumulative (mm)	1002.7	Cumulative (mm)	401.3

the row with a separation of 0.25 m. Observations were realized once every two weeks, from eight weeks before harvest. Organizations of the treatments are described in Table 3.

Control means plants without any application of fertilizer until harvest. Calcium product employed was Calcibor (12.9% w/v CaO and 2.6% w/v B) in doses of 4 L ha^{-1} (v/v = 4 L/2000 L); meanwhile, Silicon product used was NewSil (0.8% w/v Silicic acid- Si $(OH)_4$, 0.18% w/v H_3BO_3, 49% w/v Polyethylene glycol) in doses of 1.5 L ha^{-1} (v/v = 2 mL/L). Calcium and silicon were sprayed on the fruit shell and crown during nighttime, following the information about uptake and mobility of minerals after flower induction in pineapple fruit of Chen and Paull (2000) and Vásquez-Jiménez and Bartholomew (2018).

Mineral analysis

The samples' mineral content analysis of calcium, magnesium, potassium, and boron was carried out by atomic absorption spectrometry (AAS 932 Plus, GBC scientific equipment, USA). A composition of five fruits and crowns per replication was analyzed in every treatment based on the method described by Benton-Jones (2001). First, 5 mL of juice extracted from the crown leaves and the fruit flesh adjacent to the core were taken. Next, the juice was filtrated with paper to remove contaminants or residuals in the liquid sample and put in a digestion tube. After that, 5 mL of 65% nitric acid through the digestion tube walls were added and left overnight. Later, the sample was heated with a block digester at 125 °C for one hour and subsequently lifted and cooled. Thereafter, 3 mL of 30% hydrogen peroxide (H_2O_2) through the digestion tube walls were added, reheat for one hour, removed and cooled. This step was repeated three to five times until a clear filtrate was obtained. Afterwards, HNO_3 was used to prevent the filtrate from drying out (1 mL residue), warming and cooling the sample again. 5 mL of nitric acid with distilled water (1:10) were added and shake using a Vortex Shaker. Finally, the sample was transferred to a flask 25/50 mL quantitatively and pitch with distillate water creating an extract ready for mineral analysis. Results are expressed in dry basis content.

Table 3 Organization the cover treatments implemented in the research

Treatment	Characteristic
A	Control
B	Ca from ten weeks BH until harvest
C	Ca from six weeks BH until harvest
D	Si from ten weeks BH until harvest
E	Si from six weeks BH until harvest
F	Ca + Si from ten weeks BH until harvest
G	Ca + Si from six weeks BH until harvest

BH: Before harvest. Calcium and silicon doses in their respective treatments were administrated once every two weeks until harvest

Silicon content determination

Silicon content was determined in the form of silica (SiO_2) using the spectroscopy method described in Liang et al. (2015). A composition of five fruits and crowns per replication was examined in every treatment. The procedure was divided into two parts: the sample preparation implementing the autoclave-induced digestion method (AID), and the second was regarding the silica determination implementing the molybdenum blue method. The Sample preparation started with 100 mg of straw samples milled and placed to pass a 20-mesh screen and dried to a moisture content of < 10% into a 250 mL polyethene tube. After that, 2 mL 50% H_2O_2 and 4.5 mL 50% (w/w) NaOH were added. The resulting suspension in an autoclave at 138 kPa for 1 h was digested, and consequently, the digested sample was diluted to 50 mL with distilled water. Finally, the samples were ready to determine their silica content colourimetrically.

Before initiating the second part of the procedure, the following reagents were prepared: A Si standard solution of 50 mg L^{-1} Si (1000 mg L^{-1} Si standard diluted with 2.5 mM HCl to adjust the pH of the solution in the range between two and four). A 0.5 M B solution and 0.1 M B solution form H_3BO_3 (stored in plastic bottles). A stock of 0.5 M Mo solution from $Na_2MoO_4 \cdot 2H_2O$ (stored in a polypropylene bottle). A Stock of H_2SO_4 solution (0.8 M H_2SO_4 + 0.5 M B) from concentrated H_2SO_4 and H_3BO_3. A working Mo solution (0.25 M Mo + 0.4 M H_2SO_4 + 0.25 M B) freshly prepared before use by combining one volume of the stock H_2SO_4 solution and one volume of the stock Mo solution (stable for about 1 month at 5 °C). A 0.5 M citric acid (stock citric.) solution with the addition of 250 mg L^{-1} of benzoic acid as an antiseptic. A 0.1 M citric acid (working citric) solution from 0.5 M citric acid. A 1 M of tartaric acid.

The method started with 1 mL of sample solution transferred to a 50 mL volumetric flask. Thereafter, 30 mL 20% acetic acid and 10 mL ammonium molybdate solution (54 g L^{-1}, pH 7.0) were added, shook up to mix thoroughly and kept for 5 min. Immediately, 5 mL 20% tartaric acid and 1 mL of reducing solution containing 8 g L^{-1} Na_2SO_3, 1.6 g L^{-1} 1-amino-2-naphthol-4-sulfonic acid and 100 g L^{-1} $NaHSO^3$ were added. Then, the solution was adjusted to 50 mL with 20% acetic acid and waited for 30 min. To conclude, Silica was determined at the absorbance of 650 nm, measured by an automated spectrometer. Like calcium, as the fruits and crown samples' moisture content were determined beforehand, the results are expressed in dry bases.

Flesh translucency determination

Five fruits per replication in each treatment were examined at harvest time to determine the flesh translucency. The fruits were cut longitudinally in two parts for the evaluation of the severity. In addition, a subjective method was employed. The method consisted of observing the number of visible ovaries of the fruit flesh adjacent to the core, as described by D'Eeckenbrugge and Leal (2003) and Montero-Calderón et al. (2010). Previous trials demonstrated that usually, this ovary number range between 10 to 18 in a half of the longitudinal cuts of the fruit flesh, receiving the highest number the fruits with the maximum length.

Consequently, the number of ovaries turned into brown-dark colour as usually is exposed in flesh translucency was calculated (Fig. 1). Next, the fruit's severity percentage was determined in the two longitudinal cuts by accounting the number of brown-dark ovaries, divided by the number of visible ovaries. After that, the incidence was obtained by computing the percentage of fruits affected by translucency in every observation.

Evaluation by Scanning Electron Microscope—Energy-dispersive X-ray (SEM–EDX)

SEM analysis was conducted following the method used in Hu et al. (2012) on second trial samples. First, employing a tweezer, a thin piece of flesh tissue was taken (5 × 5 × 2 mm^3), focusing on the middle of the flesh. Then, before scanning, in a series of ethanol solutions, the slices were dehydrated and dried at a critical point of liquid CO_2, using a desiccator. Thereafter, the samples were mounted onto aluminium specimen stubs employing a conductive silver glue and sputter-coated with gold. Finally, SEM was processed with a scanning electron microscope (ZEISS/EVO MA 10, German) equipped with an Energy Dispersive Spectrometer (EDS), which permits a SmartEDX analysis at 15 kV. EDX analysis was carried out to reveal the calcium, potassium, magnesium and silicon weight percentage concentration in the cell wall.

Statistical analysis

Statistical analyses were performed using SPSS Version 22.0 software (SPSS Inc., Chicago, IL, USA). All data were analyzed by ANOVA of one-way. Mean significant differences at $P < 0.05$ were determined by Duncan's multiple range tests and Kruskal–Wallis test (specifically for the translucency data).

Results

Flesh translucency

The translucency incidence and severity results did not expose statistically significant differences in both trials, although the analysis of the mean values in every treatment gave important insights. Treatment E (5%) had the lowest incidence for trial one compared to treatment F, which had

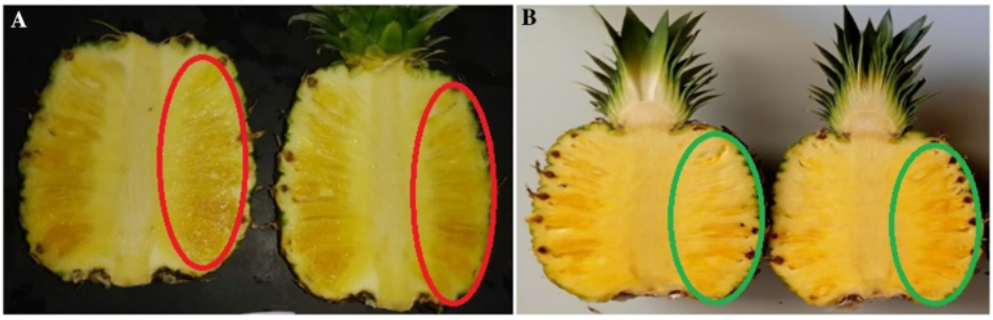

Fig. 1 Illustration of the ovary identification method in the flesh. **A** Identification of the high translucent ovary, generally with a dark colour in the flesh (red ring). **B** Ordinary recognition of the ovary in one side of the flesh for a non-translucency fruit adjacent to the core (green ring)

the most elevated one (27.50%). On the contrary, in the second trial were the treatment B (5%) and D (18.75%) the ones with the lowest and highest incidence, respectively (Table 4). The severity evidenced that the results are uncorrelated with the incidence in both trials, as the treatments with the most reduced or elevated incidence did not obtain the lowest or highest severity, respectively. The highest severity was observed in treatment F (3.47%) in trial one, linked to the most elevated incidence; nevertheless, the lowest severity was identified in treatment C (0.52%). A similar pattern happened in trial two, where the lowest severity was in the same treatment with the most reduced incidence (treatment B, 0.48%); however, the highest severity was obtained in treatment G (4.59%) (Table 4). These results demonstrate that the severity highly depends on the fruits' unique physical status, and this is not a precise index to examine the influence of any treatment implemented. In this context, the incidence appears more appropriate to consider this type of analysis.

Mineral analysis results

The minerals outcomes of the research revealed that, for the calcium content in the first trial, the treatment E got the highest result (1843.04 mg kg^{-1}), followed by the treatment F (1817.23 mg kg^{-1}), while treatment G obtained the most reduced value (1188.48 mg kg^{-1}). Likewise, for trial two, treatment E and F obtained the most elevated results (1950.32 and 2068.84 mg kg^{-1}, respectively), and G had the lowest one (1434.27 mg kg^{-1}). For the potassium content in the second trial, there were not any significant differences exposed. In trial one, treatment E (16,346.48 mg kg^{-1}) had the most elevated value, with treatment G getting the lowest result (13,502.13 mg kg^{-1}) (Table 4). Figure 2 shows the trend of calcium content during the experiment for the treatments with the highest and lowest translucency incidence in both trials. The graphic evidences a decreasing trend in the calcium content from the beginning to the end of the experiment. The decreasing trend is more remarkable in the treatments examined for trial two than those of the first trial.

Magnesium in both trials shows significant differences in the outcomes. The most representative results in trial one were observed in treatment G with the lowest value and treatment D with the most elevated outcome (988.61 and 1518.19 mg kg^{-1}, respectively). On the contrary, in trial two, the most elevated values were exposed in treatment E (1674.90 mg kg^{-1}) and F (1670.66 mg kg^{-1}), and the most reduced in treatment G (1323.53 mg kg^{-1}). In the case of the boron content, trial one exposed the most remarkable differences in treatment A and B (17.82 and 17.83 mg kg^{-1}, respectively), while the rest of the treatments obtained similar mean values, being around 14.5 mg kg^{-1} (Table 4).

The silicon content did not provide significant differences for the outcomes of trial two, although, in trial one, the most elevated result was evidenced in the treatment E (2140.01 mg kg^{-1}), whereas the rest of the treatments show similar mean results, ranging the 1500 mg kg^{-1} (Table 4). Figure 3 exposed the silicon content trend trough the experiment for the treatments with the most elevated and reduced translucency incidence in both trials. Similar to Fig. 2, this graphic exposes a decreasing trend from the beginning until the end of the research, being more remarkable in trial two. This graphic shows that the treatments implemented highly impacted the silicon level between six to two weeks before harvest because, in this period, the silicon level had its more remarkable trend variation. This finding demonstrates that the calcium and silicon applications used were more influential during this period.

Comparing the treatments' results having the lowest and highest translucency incidence with the calcium, potassium, magnesium, and silicon content in each trial, it is possible to suggest an interaction between these variables. In trial one, the treatments having the most reduced translucency incidence obtained high calcium, potassium, magnesium, and silicon content; a contrary outcome was displayed in those treatments related to the most elevated incidence. Meanwhile, in trial two, this pattern worked oppositely, having the lowest mineral content, those treatments related to the lowest translucency incidence. Although, there was no a remarkable connection for the mineral level linked to the most elevated translucency incidence in this trial (Table 4).

Crown mineral analysis results

The crown's calcium content did not display any significant differences in the values obtained for both trials. Nonetheless, the silicon content exposed significant differences in the results of the trial one and two. For trial one, silicon content was highest in treatment F (9844.82 mg kg^{-1}) and lowest in C (6101.22 mg kg^{-1}). Moreover, in the second trial, the most elevated treatment was observed in treatment B (18,999.80 mg kg^{-1}) with the lowest value in G (14,744.01 mg kg^{-1}) (Table 5).

Concerning the translucency incidence, an influence of the crown's silicon content is exposed in the results. The most elevated incidence was associated with the highest silicon content in the crown in trial one; nevertheless, the lowest silicon level was unrelated to the most reduced translucency incidence in this trial. For trial two, the highest silicon content was related to the lowest incidence

Table 4 Effect of Ca and Si treatments at different time intervals on the mineral content in the flesh of pineapple at harvest

Mineral content 1st Trial						Translucency (%)	
Treatment	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	B (mg kg^{-1})	Si (mg kg^{-1})	Severity	Incidence
A	1339.15 ± 66.11 bc	13,362.32 ± 591.89 b	1176.25 ± 57.12 b	17.82 ± 0.22 a	1564.61 ± 56.97 b	2.15 ± 0.48 a	10.00 ± 2.24 a
B	1457.54 ± 36.26 b	13,833.12 ± 519.70 ab	1216.35 ± 42.23 b	17.83 ± 0.74 a	1605.39 ± 58.24 b	1.77 ± 0.40 a	15.00 ± 3.35 a
C	1461.00 ± 55.28 b	13,683.16 ± 294.75 ab	1110.29 ± 70.86 bc	14.72 ± 0.30 b	1485.36 ± 132.03 b	0.52 ± 0.12 a	7.50 ± 1.68 a
D	1469.81 ± 32.63 b	14,795.55 ± 675.19 ab	1518.49 ± 32.30 a	14.26 ± 0.46 b	1466.99 ± 47.05 b	1.84 ± 0.41 a	15.00 ± 3.35 a
E	1843.04 ± 128.27 a	16,346.48 ± 1290.10 a	1478.25 ± 91.24 a	14.31 ± 0.82 b	2140.01 ± 113.72 a	2.17 ± 0.48 a	5.00 ± 1.12 a
F	1817.23 ± 96.27 a	14,734.35 ± 1172.62 ab	1260.18 ± 99.62 b	14.82 ± 0.42 b	1649.74 ± 182.16 b	3.47 ± 0.78 a	27.50 ± 6.15 a
G	1188.48 ± 68.41 c	13,502.13 ± 245.82 b	988.61 ± 40.31 c	15.04 ± 0.23 b	1399.61 ± 148.81 b	1.35 ± 0.30 a	17.50 ± 3.91 a
Mineral content 2nd Trial						Translucency (%)	
Treatment	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	B (mg kg^{-1})	Si (mg kg^{-1})	Severity	Incidence
A	1673.50 ± 82.61 b	11,952.01 ± 529.42 a	1435.28 ± 69.70 ab	14.05 ± 0.17 a	1735.82 ± 63.20 a	1.97 ± 0.31 a	15.00 ± 2.37 a
B	1482.06 ± 36.87 bc	11,590.44 ± 435.45 a	1369.48 ± 47.54 b	13.77 ± 0.57 a	1538.67 ± 55.82 a	0.48 ± 0.08 a	5.00 ± 0.79 a
C	1587.18 ± 60.05 bc	12,010.08 ± 258.71 a	1502.71 ± 95.91 ab	12.23 ± 0.25 b	1710.72 ± 152.06 a	3.33 ± 0.53 a	12.50 ± 1.98 a
D	1555.57 ± 34.53 bc	13,110.19 ± 598.28 a	1533.68 ± 32.63 ab	12.01 ± 0.39 b	1531.89 ± 49.13 a	3.01 ± 0.48 a	18.75 ± 2.96 a
E	1950.32 ± 135.74 a	13,830.20 ± 1091.51 a	1674.90 ± 103.38 a	12.84 ± 0.74 ab	1832.99 ± 97.41 a	2.11 ± 0.33 a	12.50 ± 1.98 a
F	2068.84 ± 109.60 a	13,319.24 ± 1060.00 a	1670.66 ± 132.07 a	13.66 ± 0.39 a	1765.40 ± 194.93 a	2.62 ± 0.41 a	11.25 ± 1.78 a
G	1434.27 ± 82.56 c	11,872.09 ± 216.15 a	1323.53 ± 53.96 b	14.19 ± 0.21 a	1487.28 ± 158.13 a	4.59 ± 0.73 a	16.25 ± 2.57 a

[a]Each value represents a mean ± standard error. Mean values in each column followed by the same lower-case letters are not statistically different by Duncan's multiple range test and Kruskal–Wallis test (for the translucency incidence and severity data) ($P < 0.05$)

[b]A (Control: No application of Ca and Si), B (Ca from ten weeks BH until harvest), C (Ca from six weeks BH until harvest), D (Si from ten weeks BH until harvest), E (Si from six weeks BH until harvest), F (Ca + Si from ten weeks BH until harvest), and G (Ca + Si from six weeks BH until harvest)

BH: Before harvest

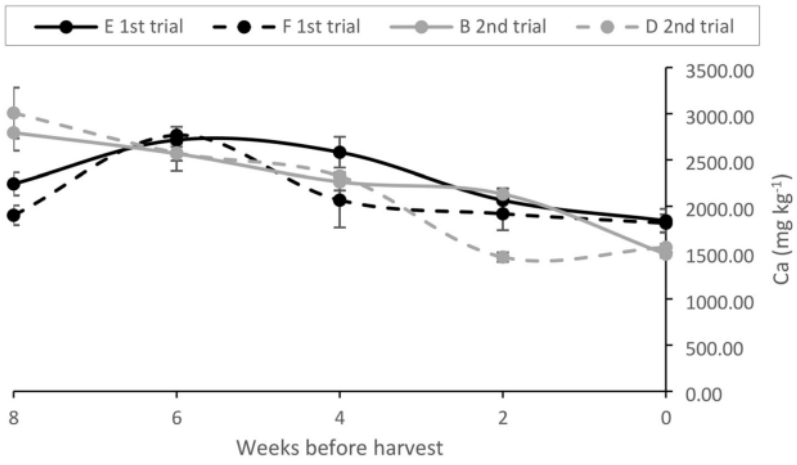

Fig. 2 Trend of the silicon content during the experiment in the pineapple flesh, for the treatment E and F in the first trial, and treatment B and D in the second trial. B (Ca from ten weeks before harvest until harvest), D (Si from ten weeks before harvest until harvest), E (Si from six weeks before harvest until harvest), F (Ca + Si from ten weeks before harvest until harvest). Values are the mean of 4 replicates, and error bars represent the standard error

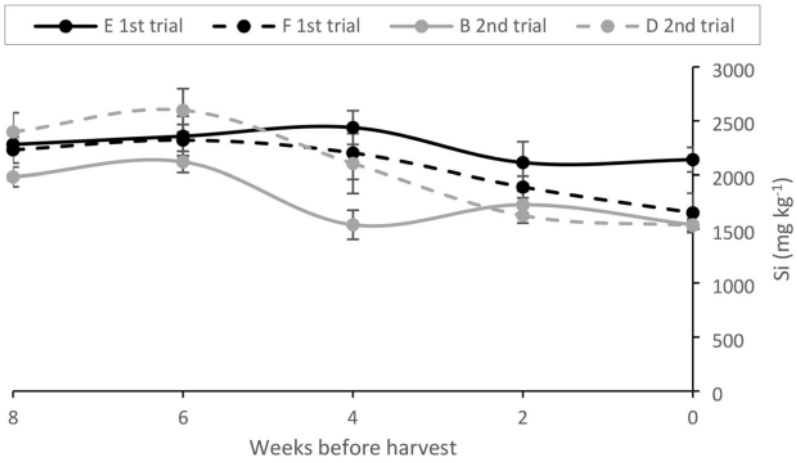

Fig. 3 The trend of the silicon content during the experiment in the pineapple flesh, for the treatment E and F in the first trial, and treatment B and D in the second trial. B (Ca from ten weeks before harvest until harvest), D (Si from ten weeks before harvest until harvest), E (Si from six weeks before harvest until harvest), F (Ca + Si from ten weeks before harvest until harvest). Values are the mean of 4 replicates, and error bars represent the standard error

of translucency, although the lowest silicon content was unassociated with the most elevated incidence.

SEM–EDX analysis and the effect on the cell wall structure

SEM analysis of the fruit flesh was carried out at harvest in the treatment A, B, and D for the second trial. These treatments were selected because those represented the control, and the lowest and highest translucency incidence, respectively. Figure 4 shows that treatment B and D maintained the cell wall integrity at harvest; on the contrary, treatment A evidenced symptoms of integrity losses in their cell wall structure. The symptoms were exhibited by clear broken holes across the cell wall area of examination. These results suggested that treatments using calcium and silicon preserve the cell wall integrity more than the control (treatment A), without fertilizer applications. On top of that, another ultra-structure cell analysis could be suggested, because treatment D, despite having the highest translucency incidence, did not expose symptoms of cell wall integrity losses. Therefore, an analysis with the possibility to identify in detail the cell wall components and layers is recommended.

Table 6 displays the EDX results of the cell wall examined for treatments A, B and D in trial two, accompanied by their translucency incidence outcomes. The results were calculated in terms of potassium, calcium, magnesium and silicon weight percentage. Potassium percentages show that is the principal weight component in the cell wall of the samples analyzed. Treatment A and D exposed a higher percentage compared to B. In the case of the calcium, treatment B obtained the highest weight percentage, which demonstrated better assimilation of this mineral in this treatment. Additionally, a hypothesis of high calcium assimilation in the cell wall linked to a low

Table 5 Effects of the treatments on the calcium and silicon content in the pineapple crown at harvest

Mineral content 1st Trial		
Treatment	Ca (mg kg^{-1})	Si (mg kg^{-1})
A	4591.71 ± 423.61 a	6297.51 ± 176.21 ab
B	5293.55 ± 421.34 a	7071.58 ± 536.18 ab
C	5201.89 ± 324.88 a	6101.22 ± 376.54 c
D	4392.43 ± 613.66 a	6402.22 ± 257.96 ab
E	4026.70 ± 332.84 a	7040.51 ± 303.86 ab
F	4270.06 ± 547.90 a	9844.82 ± 333.84 a
G	4929.90 ± 213.93 a	7251.27 ± 379.86 b
Mineral content 2nd Trial		
Treatment	Ca (mg kg^{-1})	Si (mg kg^{-1})
A	3051.46 ± 281.51 a	15,124.88 ± 423.22 b
B	3338.37 ± 265.71 a	18,999.80 ± 1440.54 a
C	3262.29 ± 203.74 a	15,661.52 ± 966.56 b
D	2884.96 ± 403.06 a	14,846.55 ± 598.19 b
E	2477.93 ± 204.82 a	16,131.85 ± 696.23 b
F	2852.87 ± 366.06 a	16,815.47 ± 570.22 ab
G	2888.47 ± 125.34 a	14,744.01 ± 772.36 b

[a]Each value represents a mean ± standard error. Mean values in each column followed by the same lower-case letters are not statistically different by Duncan's multiple range test and Kruskal–Wallis test (for the translucency incidence and severity data) ($P < 0.05$)

[b]A (Control: No application of Ca and Si), B (Ca from ten weeks BH until harvest), C (Ca from six weeks BH until harvest), D (Si from ten weeks BH until harvest), E (Si from six weeks BH until harvest), F (Ca + Si from ten weeks BH until harvest), and G (Ca + Si from six weeks BH until harvest)

[c]BH: Before harvest

translucency incidence can be proposed based on this treatment's EDX results. Furthermore, the magnesium outcomes evidenced that treatment A and B assimilated this mineral better in the cell wall than D. On the other hand, the silicon results gave the most elevated value in treatment B. These results indicated a relationship between the high silicon level in the cell wall and a low translucency incidence.

Discussion

Fruit flesh results

Because of the lack of correlation between the translucency severity and incidence, the results' discussion focuses on the incidence outcomes and their relation with the other variables studied. Severity could depend more on the unique mineral status, sugar content, and temperate

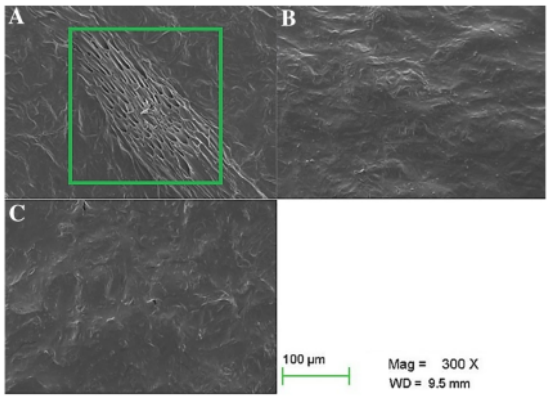

Fig. 4 Effects of the treatments implemented (A, B, D), for the second trial, on the flesh cell walls of pineapple fruit samples detected by SEM (100 µm size, 300 × of magnification). Integrity and possible discontinuities of the cell wall (green square) were examined. A (Control: No application of Ca and Si), B (Ca from ten weeks before harvest until harvest), D (Si from ten weeks before harvest until harvest)

influence of the fruit sample analyzed (Chen & Paull, 2017; Chen et al., 2009).

Flesh mineral outcomes

The occurrence of translucency decreases employing calcium fertilization during fruit development (Cano-Reinoso et al., 2021a, 2021b; Paull & Chen, 2018). Translucency appears when the fruit cannot obtain sufficient calcium, causing loss of the cell membranes' integrity, leading to leakage (Cano-Reinoso et al., 2021a, 2021b; Paull & Chen, 2018). The recommended value of calcium in the flesh should be higher than 2500 mg kg^{-1} at harvest (Paull & Chen, 2018). However, there is no sufficient evidence in the literature regarding this suggestion, as most research on calcium in pineapple focuses on its content in the leaf or soil. Furthermore, while the high calcium level in trial one was related to the lowest translucency incidence, its most elevated content in trial two did not display this connection. This fact suggests an influence and interaction of any other variables, especially agronomical or environmental, which generated this outcome.

From the agronomical point of view, the primordial influence could come from the silicon applications, as this mineral was part of the treatment employed in the research. Although there was no significant impact of the silicon content in the fruits in trial two, its superior level was linked to the lowest translucency incidence in the first trial. This approach is challenging to study due to the lack of information on silicon content in pineapple and its physiological response. An environmental approach that

Table 6 EDX analysis results of the cell wall examined and their related translucency incidence outcomes in treatments A, B and D for the second trial

Treatment	Weight (%)					Translucency
	K	Ca	Mg	Si	Total	Incidence (%)
A	75.9 ± 0.17	14.82 ± 0.08	7.36 ± 0.06	1.91 ± 0.04	100	15.00
B	69.71 ± 0.12	22.60 ± 0.08	4.4 ± 0.04	3.29 ± 0.04	100	5.00
D	78.43 ± 0.11	17.11 ± 0.06	1.9 ± 0.03	2.56 ± 0.06	100	18.75

*Each value represents a mean ± standard error. A (Control: No application of Ca and Si), B (Ca from ten weeks BH until harvest), and D (Si from ten weeks BH until harvest)

explains this mineral's physiological effects on the plant needs to be used to understand this experiment's outcomes.

Generally, it has been recognized that silicon has more effects on plants under abiotic or biotic stress (Frew et al., 2018; Majeed et al., 2019). This fact indicated that the trial one results had been influenced by an abiotic stress and the subsequent silicon response, contrary to the trial two outcomes. The rainfall could have generated this situation; the minimum monthly rainfall required for the pineapple plant range between 50 and 100 mm (Carr, 2012; Vásquez-Jiménez & Bartholomew, 2018). Data of the rainfall (Table 2) for both trials mostly exceed this range. Nevertheless, those were higher in trial one, and the accumulation of three months was highly elevated compared to trial two (1002.7 mm, 401.3 mm, respectively). High rainfall, especially in a crassulacean acid metabolism (CAM) plant, adapted to live under drought conditions could generate waterlogging stress (Carr, 2012; Vásquez-Jiménez & Bartholomew, 2018).

The reduction in the levels of O_2, and the decrease of the hydraulic conductivity are considered some of the primordial plant responses to waterlogging (Muhammad Arslan Ashraf, 2012; Zhou et al., 2020). Low hydraulic conductivity could generate a decrease in the mineral uptake by the fruit (Hocking et al., 2016; Vásquez-Jiménez & Bartholomew, 2018). On the other hand, the production of reactive oxygen species (ROS) are the result of the reduction of O_2 (Muhammad Arslan Ashraf, 2012; Zhou et al., 2020). ROS can perturb several cellular metabolic processes of the plants, mostly related to the cell wall degrading and constitution, primordial factors in controlling translucency in pineapple (De Freitas & Resender Nassur, 2017; Paull & Chen, 2018).

The increase of antioxidant metabolic activity and ROS's reduction have been linked to proper fertilization with silicon (Frew et al., 2018; Majeed et al., 2019). Moreover, silicon fertilization has been associated with alterations in mineral uptake and accumulation of macronutrients (P, K, Ca and Mg) (Frew et al., 2018; Majeed et al., 2019). This information demonstrates why the results of the translucency incidence varied in each trial. Evidently, due to the waterlogging stress, the triggering of the silicon metabolic activity occurred. Due to the waterlogging stress, treatment E triggered the silicon influence on the plant, enhancing the mineral uptake and lowering translucency incidence, contrary situation when treatment F was implemented. Calcium mixed with silicon evidenced a possible negative interaction in this situation. Furthermore, when there was no stress, as in trial two, treatment B was enough to reduce the translucency incidence, despite not having a high calcium or silicon content, while treatment D (employing silicon) caused the most elevated translucency incidence. Figure 2 and 3 displayed that between six to two weeks before harvest, the calcium and silicon content increased or was steadier for the treatments with the lowest incidence of translucency (E and B, respectively); there may be an increase in the fruit's ROS production during this time, speeding up the cell wall degrading. Therefore, the fruit's elevated uptake of calcium and silicon to mitigate this negative physiological impact, especially in trial one.

On the other hand, potassium has been associated with influencing fruit weight, total soluble solid content, and acidity in pineapple (Vásquez-Jiménez & Bartholomew, 2018). Regarding physiological impacts, potassium has been found to increase the aquaporin activities in plants, resulting in an improvement of the hydraulic conductivity (Kanai et al., 2011; Wang et al., 2013). This condition causes an improvement in the mineral uptake and photoassimilates to the fruit, improving calcium assimilation, and affecting translucency. Also, this mineral has been linked to a reduction of ROS by increasing the photosynthetic CO_2 fixation and inhibiting the transfer of photosynthetic electrons to O_2 (Srivastava et al., 2020; Wang et al., 2013). This information demonstrated why treatment E, having the lowest translucency incidence in the first trial, had the highest potassium content. Interaction with the silicon may increase the signalling of the K^+ ion and mineral uptake by the fruit, reacting to the plant's waterlogging stress. Further, as the plant sensed no stress in the second trial, an influence or interaction that affected the translucency between the silicon, calcium, and potassium content was undisplayed.

Like potassium, magnesium in pineapple has been related to influences on chlorophyll concentration, photosynthesis, and growth (Vásquez-Jiménez & Bartholomew,

2018). Magnesium has been reported to activate more enzymes than any other mineral in plants, like the protein kinases (Waraich et al., 2011; Xie et al., 2020). Together with the calcium, these enzymes play an essential role in stabilising the cell membranes and permeability (De Freitas & Resender Nassur, 2017). This fact could explain why magnesium could be positively correlated to a reduction in the translucency incidence through its effect on the fruit cell walls. Consequently, this information clarifies why treatment E gained two of the highest magnesium level outcomes in the first trial. The silicon implementation in this treatment increased the magnesium content, permitting the reduction of translucency. On the contrary, for trial two, as there was no influence of the waterlogging stress, the interaction previously described was not displayed.

Furthermore, boron in pineapple plant has been found to forming and strengthening cell walls (Vásquez-Jiménez & Bartholomew, 2018). Boron ions (B^{3+}) are cross-linked to the rhamnogalacturonans (RG), a complex class of polysaccharides, helping to rigidify the cell wall matrix (Lampugnani et al., 2018). Because of these associations, several authors have suggested that proper fertilization and mineral uptake of boron could support the plant in case of any abiotic stress impact, due to its cell wall influences (Waraich et al., 2011). Although a higher or lower boron content was unlinked to the most elevated or reduced translucency incidence in any of the trials, further research could be performed on this matter.

Crown results

The only reports available regarding the crown's influence on translucency associated this to a competition with the fruit for photoassimilates (Chen & Paull, 2017; Paull & Chen, 2015). Concerning the crown's mineral status, despite the lack of information, the results obtained, especially for the calcium content, are similar to those established for the plant leaves (Vásquez-Jiménez & Bartholomew, 2018). Moreover, the differences between both trials' outcomes demonstrated that, for any foliar application of minerals in pineapple, the crown is a crucial organ that needs further analysis. These results proved that there is mineral absorption enough to influence the quality of the fruit.

Foliar analysis suggests that the calcium requirements for MD2 cultivar are between 2500 and 3000 mg kg^{-1}, in one-third of the longest plant leaf (dry bases) (Vásquez-Jiménez & Bartholomew, 2018). On top of that, it has been stated that habitually in MD2 cultivar, it is complicated that tissue concentration of calcium exceeds 4000 mg kg^{-1} (Vásquez-Jiménez & Bartholomew, 2018). Despite not providing statistically significant differences in both trials, the calcium content was in the recommended range, and the results of trial one exceed the value of 4000 mg kg^{-1} (Table 5). This fact exposed that the crown's calcium content was higher than the flesh (Table 4). Furthermore, the crown's calcium outcomes did not influence the translucency incidence. Also, the differences in values between both trials could be associated with the waterlogging stress impacting the crown physiology.

Examinations of silicon content in MD2 leaf suggest that an adequate level would be around 3000 mg kg^{-1} or higher (in one-third of the longest plant leaf, dry bases) (Vásquez-Jiménez & Bartholomew, 2018). However, due to the lack of research on this mineral in pineapple, the critical tissue levels require future confirmation (Vásquez-Jiménez & Bartholomew, 2018). The outcomes of this mineral in the crown were more superior than the minimum recommended for the leaf. The results displayed in both trials exhibited a negative interaction with the outcomes of the flesh.

For the first trial, the highest silicon content in the crown in treatment F was linked to the highest translucency incidence, opposite to the flesh. On the contrary, in the second trial, the most elevated silicon content in the crown in treatment E was associated with the lowest translucency incidence, although for this trial in the flesh, there was no significant difference exposed. Besides, the silicon content of trial one was much lower compared to trial two. These results suggest that the flesh's silicon is more necessary when there is waterlogging stress than in the crown. Silicon could be more susceptible than calcium to photoassimilates competition and absorption by sink organs, especially when no abiotic stress impacts the plant. Nevertheless, the calcium and silicon mineral influence on the crown physiology needs more investigation.

SEM–EDX analysis results

Analysis of the SEM results in trial two shows that because of the no implementation of calcium and silicon fertilization in treatment A, a discontinuity and disruption in the cell wall constitution was occurred. This circumstance generated a high translucency incidence in this treatment. Nonetheless, treatment D obtaining the most superior translucency did not display that physical characterization. This affirmation suggests that the normal cell wall breakdown exhibited in the translucency could be exposed in another view or layer of the cell wall, which was not detected by the SEM analysis. Hemicelluloses represent the essential structural component of the primary cell wall in pineapple (41.8%), followed by cellulose (33.6%) and pectin (21.2%) (Ding & Syazwani, 2016). This information demonstrated the need for a detailed examination and description of the hemicellulose in the cell wall, regarding translucency in the future. As described previously,

physiological disorders like translucency can be linked to ROS increase causing cell wall degrading and instability (Muhammad Arslan Ashraf, 2012; Zhou et al., 2020). Therefore, ROS like superoxide (O^{2-}), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH) production in treatment D should be higher than treatment A and the lowest in treatment B.

EDX results demonstrated that despite the lower calcium content in the mineral analysis, treatment B had the highest weight percentage of calcium. This situation means this treatment permitted the higher assimilation of calcium ions (Ca^{2+}) into the cell wall. It has been previously reported that the assimilation of calcium in the cell wall places a primordial role in pineapple, affecting the flesh firmness positively and decrease the translucency incidence (Cano-Reinoso et al., 2021a, 2021b; Paull & Chen, 2018). These outcomes suggest that examining the calcium accumulated in the cell wall is more effective than a mineral determination in the fruit flesh to establish a translucency incidence influence. Treatment B was also described by the most elevated weight percentage of silicon in the cell wall. Similar to the calcium in the second trial, silicon mineral analysis in the flesh did not expose a high level for this treatment; nonetheless, its ion assimilation was the highest compared to the treatment A and D. These outcomes exposed that, without abiotic stress, silicon still influence the fruit's physiology; however, it is more influential at the ultra-cellular level. The high potassium content displayed in all the EDX analysis of the treatments is related to the cell wall polysaccharides matrix. As mentioned previously, K is vital for carbohydrates and protein synthesis (Oosterhuis et al., 2014; Wang et al., 2013). Many of those carbohydrates are part of the constitution of this polysaccharide matrix of the cell wall (Oosterhuis et al., 2014; Wang et al., 2013). Therefore, it was expected that the EDX analysis in each treatment examined delivered a high weight percentage of potassium. In the case of magnesium, treatment B, despite not having the highest magnesium weigh percentage, shows that an interaction with the silicon at this cellular level could trigger the magnesium's role in the stabilization of the cell membranes. This positive interaction caused that this treatment had the lowest translucency incidence in the outcomes. Treatment A despite having the highest magnesium level obtained the lowest silicon weight percentage; moreover, treatment D had the lowest magnesium weight percentage and consequently, the highest translucency incidence, possibly because of the lack of interaction with silicon at cellular level.

Conclusion

Calcium and silicon foliar fertilization after flowering affected the pineapple mineral status and flesh translucency. Treatment E (Si from six weeks BH until harvest) and B (Ca from ten weeks BH until harvest) delivered the best results regarding the most effective reduction of translucency incidence in trials one and two, respectively. Meanwhile, treatment F (Ca + Si from ten weeks BH until harvest) in trial one and treatment D (Si from ten weeks BH until harvest) in trial two displayed the worst performance, increasing the translucency incidence. In trial one, treatment E had the highest potassium, calcium, magnesium and silicon accumulation to cause the lowest translucency incidence; however, this interaction was not presented in the outcomes of the second trial, essentially in the treatment B. Regarding the crown, the silicon mineral content in both trials affected the translucency incidence. The incidence of translucency was affected by a waterlogging stress in the first trial. Besides, it was determined that silicon positively influenced the mineral accumulation in pineapple when this stress affected the plant.

EDX examinations of the second trial outcomes exposed that the assimilation of minerals into the cell wall could be more primordial to translucency than the mineral content in the flesh. Treatment B obtained the most elevated weight percentage of calcium ions assimilation in the cell wall and the lower translucency incidence. An influence of silicon was also evidenced in this ultra-cellular level. An interaction between calcium, magnesium, and silicon in the cell wall related to translucency was proposed. Finally, more experiments should be done on calcium and silicon influences on pineapple fruit and crown physiology and their relation with flesh translucency.

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Data availability Authors declare that the data and materials of this manuscript have been collected and provided with integrity and those can be fully provided in case of a further enquiry.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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2.Effect of calcium and silicon fertilization after flowering on pineapple mineral status and flesh translucency

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