The Relationship of Glucomannan, Oxalate, and Crystal Development in Porang Tuber (*Amorphophallus muelleri* Blume)

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Abstract

Chemical compounds are presumed to interact with each other and potentially affect the crystal development of porang tubers. This study focused on glucomannan and calcium oxalate (CaOx) as chemical compounds found in porang. Crystal development was analyzed by microscopic observation. The tuber tissue was used for microscopic slides and was harvested two weeks before the plants lost their leaves, when the plants lost their leaves, and two weeks after the plants lost their leaves. The CaOx and glucomannan content was measured using a modified extraction method. The increasing of CaOx content tends to increase the number of CaOx crystals. The analysis showed that the crystal density could affect the increasing of tuber weight possibility. There was a development process in raphide crystals that showed by different sizes at three harvest times, which the others were not. The results also indicated that glucomannan might influence the crystal density and the CaOx content. It can be concluded that glucomannan can potentially induce CaOx synthesis and crystal count increase.

Keywords: Caox, Crystal, Development, Glucomannan, Interact, Porang.

INTRODUCTION

Porang (*Amorphophallus muelleri* Blume) is a tropical plant the tuber contains glucomannan [1]. Glucomannan is included in the polysaccharide group, which is having many benefits. It can be processed into a dietary supplement that leads to blood sugar reduction, fat reduction, cancer suppression, and constipation and can be used as an emulsifier [2–6]. However, the utilization of porang tuber to produce glucomannan still needs to be improved. It is because porang tuber also contains calcium oxalate compound and CaOx crystals. The calcium oxalate compound and its crystals cause kidney problems, allergies, and irritation [7,8,9]. According to the previous reports, four primary CaOx crystals are found in porang tuber, i.e., raphide, druse, styloid, and prism [10,11].

Genetic and environmental factors influence glucomannan content, calcium oxalate content, and crystal density. Based on the previous study, the tuber's chemical composition is usually affected by the growth factor, maturity stage, species, and storage method [12]. The dynamic of glucomannan content in porang is usually caused by plant age, harvest time, seeding material, and storage times [13,14,15]. Oxalate content in plants is significantly influenced by genetics and environmental factors [16,17]. The oxalate formation and the combination of genetic and environmental factors also play a role in determining the number, shape, size, and function of CaOx crystals. In addition, the formation of CaOx crystals is also related to Ca transport and regulation, oxalic acid biosynthesis, and defense [18].

There are many reports on factors affecting glucomannan content, calcium oxalate, and crystal density. However, there are still no reports that specifically describe the interaction between chemical compounds found in porang tuber. Therefore, it is necessary to observe the correlation between glucomannan accumulation with calcium oxalate compounds and their crystals. This information is expected to be useful for farmers to determine the porang tuber with the highest levels of glucomannan and lowest oxalate.

MATERIAL AND METHODS

Experimental Site

Tubers were obtained from second-growing period plants in the Sumberbendo area, Saradan District, Madiun Regency, East Java. The tuber was planted to bring out third-growing period plants. Nine tubers were planted in each polybag with an average weight of 0.9-1.2 kg and a diameter of 15-16 cm. Tuber's planting was carried out until the end of the vegetative phase. Tubers were harvested after determining the harvest time (Fig. 1). Harvest time was determined at different times, i.e. (i) two weeks before the plants shed their leaves (R_0-2), (ii) when the plants shed their leaves (R_0), and (iii) two weeks after the plants shed their leaves (R_0+2).

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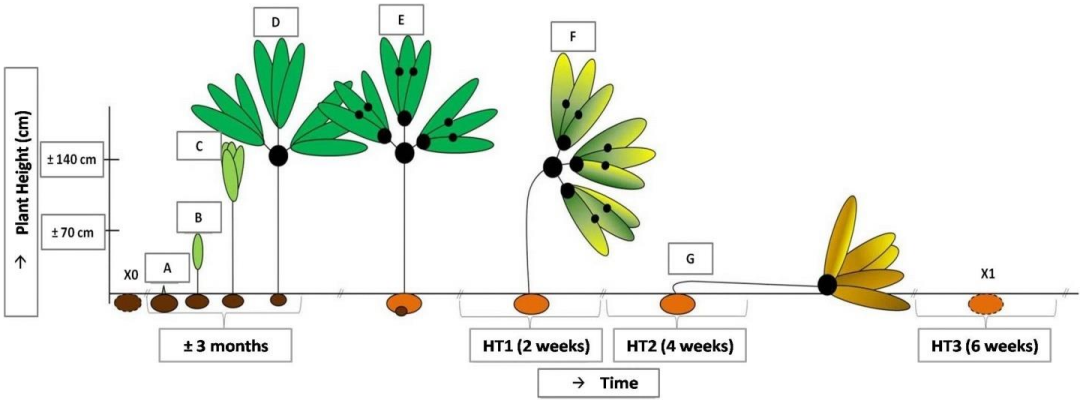

Figure 1. *Amorphophallus muelleri* growth pattern. (A). Tuber beginning to sprout; (B). shoots begin to elongate; tuber begins to shrink; (C). plant leaves begin to open; (D). The leaves of the plant have opened up, the bulbil starts to appear in the main branch, and the tuber is shrinking because its food reserves are used for growth; (E). Bulbil begins to appear in other branches, and the new tuber has formed; (F). The tips of the leaves begin to turn yellow; the petioles begin to wilt; (G). The plant is shed; X0: Dormancy phase in the second growing period tuber; X1: Dormancy phase in the third growing period tuber; HT: Harvest Time

Glucomannan Extraction

Glucomannan extraction used the modified method of Tartirat and Charoenrein [19] by changing the incubation temperature and precipitation solution. Fresh tubers were 30 g grated and were crushed using a mortar and pestle until the texture was smoother than before. Afterward, it was dissolved in 200 mL of 0.3% $Al_2(SO_4)_3$ solution. The mixture was incubated at 55°C for 15 minutes. During the incubation process, the mixture was stirred using a glass stirrer. The mixture was diluted by adding distilled water and then filtered using a chiffon cloth. The filtrate was centrifuged at 1500 rpm, at 25°C, for 30 minutes. The pellets were removed, and the supernatant was added with 95% IPA (Isopropyl alcohol) in a ratio of 1: 1 to coagulate glucomannan. The mixture was stirred occasionally using a glass stirrer. Glucomannan which was stuck on a glass stirrer, was set aside on filter paper (Whatman). Thereafter it was compacted. Later on, the compacted glucomannan was soaked again in 95% IPA. This stage was conducted to prevent browning on its surface. The remaining glucomannan in the mixture of 95% IPA and the supernatant was filtered using filter paper. The obtained glucomannan was dried at 45°C overnight to reduce the water content of the glucomannan. The glucomannan content in the tuber was determined using the formula [20]:

$$\text{Glucomannan content } \left(\frac{g}{g}\right) = \frac{A}{BK_2} \quad \text{.....1}$$

Description:
A = the weight of the glucomannan obtained (g)
BK = tuber dry weight (g)

Calcium Oxalate Analysis

Analysis of CaOx content used the modified method from Iwuoha and Kalu [21] by changing the sample type, centrifugation time, incubation process, and concentration of chemical material used in the titration process. The method contains three stages, i.e., (1) the digestion process, (2) oxalate precipitation, and (3) permanganate titration. The digestion process was begun by grating 10 g of the fresh tuber. The grated tuber was dissolved in distilled water 190 mL, and then it was added HCl 6 N 10 mL. Afterward, the suspension was heated at 100°C for one hour. Later on, it was cooled. After the cooling process, distilled water was added to the suspension until the volume reached 250 mL. The suspension was subsequently filtered using Whatman paper.

The oxalate precipitation process used four drops of methyl red added to the filtrate. After that, a few drops of NH_4OH were added until the pH reached 4-4.5. The filtrate was reheated to 90°C then it was cooled. The filtrate was filtered again using Whatman paper to remove precipitates containing Fe (iron) ions. The filtrate was heated, and then it was added $CaCl_2$ 5% 10 mL. The filtrate was homogenized with a magnetic stirrer to make it homogeneous. Later it was centrifuged at 2500 rpm for 10 minutes. The supernatant was decanted, and the pellet was dissolved using H_2SO_4 20% 10 mL.

In the permanganate titration process, 10 mL of pellets were dissolved with H_2SO_4 20 mL. Hereafter it was added to distilled water until the volume reached 100 mL. Later the solution was heated to almost boiling. After that, the solution was titrated using standardized $KMnO_4$ 0.1 N.

CaOx (g) content was calculated using the formula 2 [21], while the determination of calcium oxalate content in the tuber was calculated using the formula 3 [22] below.

$$CaOx\ Content\ (g) = \frac{V_{KMnO_4} \times M_{KMnO_4} \times 5 \times ME \times DF \times 100}{m_f \times 1000} \quad \text{.....2}$$

Description:

V_{KMnO4} : Volume of $KMnO_4$ (mL)
M_{KMnO4} : Molarity of $KMnO_4$
ME : molar equivalent of $KMnO_4$
DF : dilution factor
m_f : mass of fresh tuber

$$\text{Calcium oxalate in tuber } \left(\frac{g}{g}\right) = \frac{C}{BK_2} \quad \text{.....3}$$

Description:
C = mass of obtained calcium oxalate (g)
BK = tuber dry weight (g)

Preparation and Analysis for Microscopic Observation

The tissues for microscopic observation were obtained from the edge and center of the tuber using the modified tissue-clearing from Ilarslan *et al.* [23] by modifying the tissue-clearing agent. The first step was sampling by making tissue slices from the edge and center part of the tuber. The tissue slices were obtained using a microtome with a thickness of ± 10 µm. Tissue slices were immersed in NaOH 5% to remove the chlorophyll. During the immersion process, it was incubated in an oven at 37°C for 24 hours to accelerate the penetration of the substance into the tissue. Furthermore, it was immersed in a commercial sodium hypochlorite solution of 50% for one hour to purify the plant tissue. Later, the tissue slices were washed using running water to clean the remaining sodium hypochlorite solution. The tissue slices were subsequently immersed in the stratified concentration of ethanol solution, i.e., 30%, 50%, 70%, and 80%, each for 10 minutes. Thereafter it was soaked with ethanol 100% for 5 minutes. After soaking with ethanol, the tissue slices were placed on a glass object dripped with a Hoyer. The last step covered it with a glass cover, and it was observed using a microscope (Olympus CX31 type). CaOx crystals density was calculated using the following formulas.

$$The\ total\ density\ of\ CaOx\ crystals\ per\ slide\ (Sn) = \frac{(\sum total\ CaOx\ crsytals)/3}{field\ of\ view\ (cm^2)} \quad \text{.....4}$$

$$The\ total\ density\ of\ CaOx\ crystals\ per\ replicate\ (R) = \frac{(S_1 + S_2 + \cdots + S_n)}{n_R} \quad \text{.....5}$$

$$The\ total\ density\ of\ CaOx\ crystals\ per\ harvest\ time(T) = \frac{(R_1 + S_2 + \cdots + R_n)}{n_T} \quad \text{.....6}$$

Description:
n_R = the number of slides made per replicate
n_T = the number of slides made per harvest time

Data Analysis

The CaOx crystal density and size data were derived from observing slices of the porang tuber tissue at different harvest times. It was performed in three replications. The One-Way ANOVA test was implemented using SPSS Statistics 17.0 software to determine the effect of the harvest time on the total density and size of CaOx crystals. If a significant effect were found, this test would be followed by the Tukey Test α 0.05 [24]. A correlation test was conducted to determine the relationship between glucomannan content, CaOx, and crystal density in tuber weight. The tuber weight referred to in this study is the dry weight of the porang tuber.

RESULTS AND DISCUSSION

The glucomannan content, CaOx content and crystal density had the highest content and density found in the tuber harvested when the plants shed. However, this accumulation tended to decrease after the plants shed. It showed that the higher the glucomannan content, the higher the CaOx content and crystal density. From this phenomenon, glucomannan content, CaOx content, and crystal density were directly proportional (Table 1).

The correlation test results in the absence of a controlling variable showed a strong positive correlation between glucomannan content and CaOx content and a weak correlation between glucomannan content and crystal density, i.e., 0.626 and 0.497. However, the correlation between glucomannan content and CaOx content and crystal density of CaOx was not significant, i.e., 0.071 and 0.174.

Table 1. Dynamic of Glucomannan, Calcium Oxalate, and Crystals in porang tuber

No	Harvest time	Tuber weight (g)	Tuber dry weight (g)	Glucomannan content (%)	Calcium oxalate content (%)	Crystal density ($10^3 cm^{-2}$)
(1)	R_0-2	338.33 ± 12.58	64.80 ± 5.31	23.26 ± 1.08	9.56 ± 2.60	17.82 ± 9.66
(2)	R_0	881.67 ± 345.41	160.05 ± 61.51	29.11 ± 4.57	15.98 ± 0.59	40.02 ± 16.55
(3)	R_0+2	606.67 ± 204.29	125.32 ± 30.56	24.44 ± 2.09	10.88 ± 2.16	29.90 ± 11.24

Notes: (1) R_0-2: 2 weeks before the plants shed their leaves, (2) R_0: when the plants shed their leaves, and (3) R_0+2: 2 weeks after the plants shed their leaves.

Table 2. The correlation between glucomannan, CaOx, and crystal density in porang tubers at three different harvest times

Control Variables			CaOx_ Content	Glucomannan _Content	Crystal _Density	Tuber _Weight
-none-[a]	CaOx_ Content	Correlation	1.000	.626	.578	.641
		Significance (2-tailed)		.071	.103	.063
		df	0	7	7	7
	Glucomannan _Content	Correlation	.626	1.000	.497	.337
		Significance (2-tailed)	.071		.174	.375
		df	7	0	7	7
	Crystal _Density	Correlation	.578	.497	1.000	.443
		Significance (2-tailed)	.103	.174		.232
		df	7	7	0	7
	Tuber _Weight	Correlation	.641	.337	.443	1.000
		Significance (2-tailed)	.063	.375	.232	
		df	7	7	7	0
Tuber _Weight	CaOx_ Content	Correlation	1.000	.568	.427	
		Significance (2-tailed)		.142	.292	
		df	0	6	6	
	Glucomannan _Content	Correlation	.568	1.000	.412	
		Significance (2-tailed)	.142		.311	
		df	6	0	6	
	Crystal _Density	Correlation	.427	.412	1.000	
		Significance (2-tailed)	**.292**	**.311**		
		df	**6**	**6**	**0**	

After the tuber weight variable became the controlling variable, there was a decrease in the correlation between glucomannan content on CaOx content and crystal density, i.e., 0.568 and 0.412. It indicated that the tuber weight factor influenced the positive correlation between glucomannan content, calcium oxalate content, and crystal density. The high content of glucomannan would increase the tuber's weight, leading to an increase in the synthesis of CaOx and CaOx crystals (Table 2).

The correlation between glucomannan content and calcium oxalate content, and crystal density had a determinant value, i.e., 39.2% (R2=0.392) and 24.7% (R2=0.247) (Fig. 2). The 39.2% of CaOx content and 24.7% of crystal density were affected by the presence of glucomannan content. Previous research revealed that konjac glucomannan is related to calcium ions (Ca) [25]. The low glucomannan content prevents the aggregation of CaOx monohydrate, increases ion concentration, reduces the number of CaOx crystals, and inhibits its growth.

The CaOx monohydrate crystals are found when the glucomannan content is low and generally are round and blunt. A different phenomenon occurs when the glucomannan content is high. A high glucomannan content triggers the synthesis of CaOx monohydrate crystals which appear in irregular or sheet-like forms. Gouveia *et al.* [26] also stated that there was a significant relationship between oxalate content, carbohydrate metabolism, chlorophyll content index, and protein synthesis.

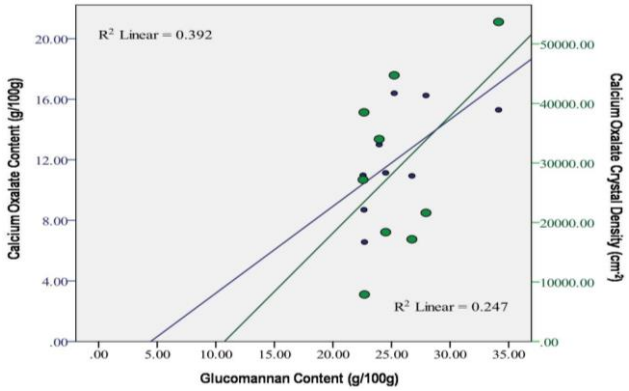

Figure 2. The correlation between glucomannan content on calcium oxalate content and crystal density

Total Density of CaOx Crystals in Porang Tuber

There was no significant difference between the total density of CaOx crystals in the porang tuber at three different harvest times. It was known from the significance value greater than α (0.05), i.e., 0.496. The total density of CaOx crystals in porang tuber harvested when the plants were shedding their leaves tended to be higher than those found in porang tuber before and after the plants shed their leaves, i.e., 40.020 ± 16.554 crystals.cm^{-2} (Fig. 3). This was assumed to be due to the influence of harvest time or harvest intervals of porang tuber. There is an assumption that the activity of glyoxylate synthesis, a precursor of oxalate formation, also increases as plants mature. Therefore, the oxalate content in adult plants also increases. The oxalate produced from this synthesis pathway had a relatively high content. It accumulated in several plant organs, one of which was a tuber.

The result agreed with Burrows and Tyrl [27], who stated that oxalate concentrations increased with plant maturity. The oxalate concentration has the highest content when the plant gets old and dries up. Jiang *et al.* [28] also supported the theory, who stated that photosynthetic activity increased in mature plant leaves. It increased gradually and reached a maximum level when the leaf reached its maximum development stage. This opinion is also supported by the research from Contreras-Padilla *et al.* [29], which shows that there are differences in CaOx content at different maturity phases in *Opuntia ficus-indica* (nopal pads). It is also mentioned in the research report that CaOx content is higher in older plants than in younger ones [29]. In contrast, Bernardino-Nicanor *et al.* [30] study showed that the older the plants, the lower the CaOx content.

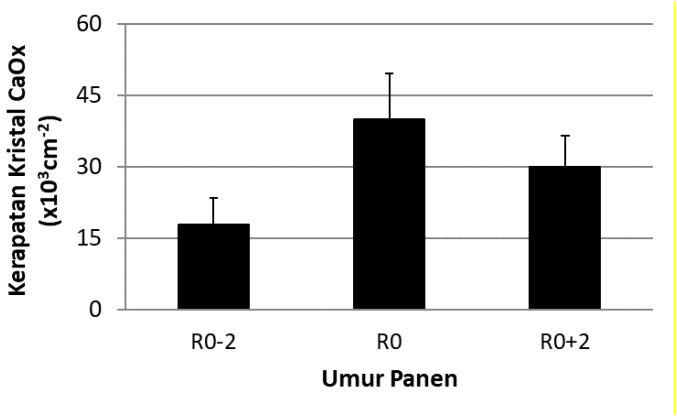

Figure 3. The total density of CaOx crystals in porang tuber at three different harvest times. Note 1). R_0 – 2 : two weeks before the plants shed their leaves (2). R_0 : when the plants were shedding their leaves (3). R_0 + 2: two weeks after the plants shed their leaves

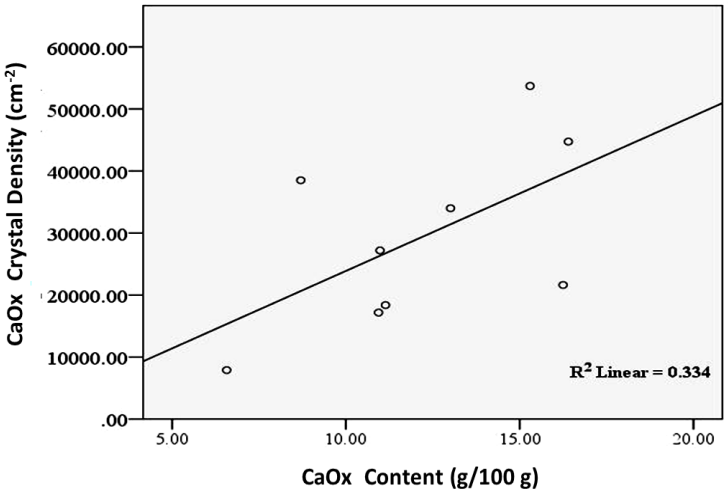

Figure 4. Relationship between CaOx content and CaOx crystal density in porang tuber in the third growth period

An increase in photosynthetic activity is thought to increase photorespiratory activity. An increase in the results of photorespiration can increase the synthesis of glyoxylates, which is a precursor to oxalate compounds [31,32]. The resulting glyoxylate is the primary precursor for oxalate formation in plants. If the oxalate binds to certain levels of Ca obtained from the environment, a reaction will be formed, resulting in precipitation in special cells, i.e., idioblasts. The precipitation is in the form of CaOx crystals.

Based on the results of the correlation test in this study, the content of CaOx was strongly correlated with CaOx crystal density. It was known from the correlation value greater than 0.5, i.e., 0.578. These results indicated that the higher the calcium oxalate content, the higher the CaOx crystal density found in the tuber (Fig. 4). The strong correlation between the CaOx content and the density of CaOx crystals is caused by the CaOx compounds forming the CaOx crystals. According to Franceschi and Nakata [18], Libert and Franceschi [31], and Cao [33], oxalate compound, which plants produce, plays a role in the regulation of calcium (Ca) in plants. The oxalate compound that binds calcium (Ca) is obtained from the absorption of nutrients in the environment. The reaction produces deposits in special cells called idioblast cells. Deposits from the reaction between oxalate and calcium (Ca) are called CaOx crystals.

Although there was a strong correlation between the CaOx content and crystal density, the correlation was not significant because it was greater than α value (0.05), i.e., 0.103 or a determination value of 33% (R2 = 0.334) (Figure 4). So, it can be said that 33% of the density of CaOx crystals was influenced by the CaOx content. The number of crystals per unit area indicates crystal density. Based on the results of this study, the calculation of determination showed that the number of crystals in the tuber, which was 33%, was influenced by the constituent material, i.e., calcium oxalate, whereas the rest, 67%, by the other factors, such as crystalline adhesives and protein matrix. The protein matrix of CaOx crystals in *Pistia stratiotes* weighs 55 kD, 60 kD, and 63 kD [34].

Based on the results of this research, the density of CaOx crystals and the weight of porang tuber have a weak correlation value, i.e., 0.443. In addition, a weak correlation is also evidenced by a significance value greater than α (0.05), i.e., 0.232 or a determination value of 19.6% (R2 = 0.196) (Fig. 5). Therefore, it can be said that the weight of the tuber influences the crystal density of CaOx 19.6%. The relationship between CaOx crystal density and porang tuber weight was also explained by Nurlaila [35] through the result of a regression test. Based on these test results, CaOx crystal density did not affect the increase in weight of porang tuber.

In addition, the abiotic and biotic factors affect the formation of CaOx crystals. The abiotic factor is the content of calcium (Ca) ions in the growing medium, while the biotic factors are pests and fungal attacks. In this study, some tubers harvested showed the symptoms of pathology caused by the pests and fungi and thus might decrease or inhibit the synthesis of CaOx crystals.

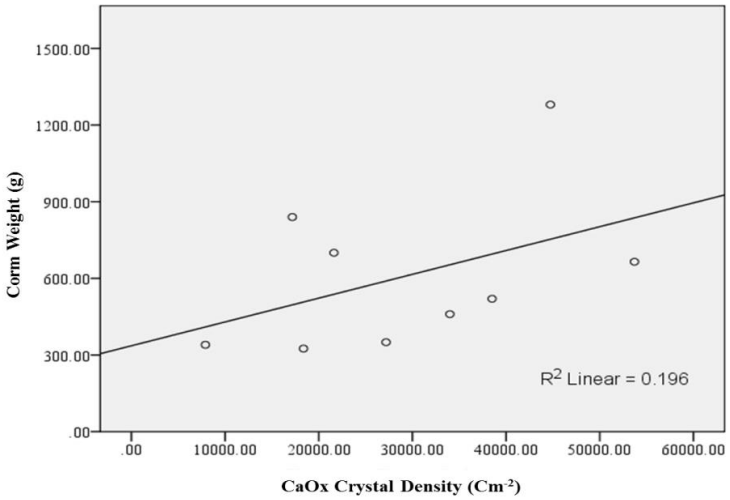

Figure 5. Relationship between CaOx crystal density and weight of porang tuber in the third growth period

Calcium is considered one factor that influences CaOx crystals' formation. It is because calcium is one of the constituent components of the crystal. In research by Mazen *et al.* [36], it was found that Ca levels affected the size and number of CaOx crystals in plants. The size and number of CaOx crystals increased along with the increase in Ca levels in the planting medium. The finding similar to Guggiari *et al.* [37] and Faheed *et al.* [38], on a positive correlation between calcium levels found in nutritional media and the formation of CaOx crystals in plants.

The higher the absorption rate, the more calcium accumulates in plants. Calcium that accumulates in plants can bind to oxalic acid. The binding of calcium and oxalate will produce crystalline deposits in idioblast cells [18,39,40]. Meanwhile, oxalate content is significantly related to carbohydrate metabolism, chlorophyll content index, and protein synthesis [26].

Based on previous research, pest attacks can induce the formation of CaOx crystals. CaOx crystals play a role in self-defense mechanisms against insects [41]. Dissolved oxalic acid also plays a role in defense against sucking insects such as planthoppers and aphids. This finding is also supported by Cote´and Gibernau [42], who stated that CaOx crystals in some plants from Family Araceae protect plants from insect predators. It has also been revealed that there is a protective mechanism between raphide crystals and cysteine proteases that inhibits growth and causes the death of larvae by perforating barrier structures such as membranes, epithelium and cuticles to increase the toxicity of bioactive factors [8].

Fungi can potentially increase the production of CaOx crystals [37]. The statement was also supported by Uloth *et al.* [43], who stated that many CaOx crystals were found on the surface of the *Brassica carinata* stem in the early stage of the *Sclerotinia sclerotiorum* infection. The CaOx crystals formation on the surface of cortical cells support the theory that the function of oxalic acid is chelating calcium ions from the cell wall. This research also shows that the interaction between *Brassica carinata* and *Sclerotinia sclerotiorum* produced various CaOx crystal morphologies. However, in addition to increasing the production of crystals, fungi could be one of the causes of decreased crystal count in plants [37]. It happens because fungus produces several acids, one of which is oxalic acid. The oxalic acid produced is assumed to come from the crystal decomposition process.

Development of CaOx Crystals Size in Porang Tuber

Based on the observation, four primary crystal forms were found in the tuber, i.e., druse, raphide, styloid, and prism (Fig. 6). The significant difference between the crystal sizes of single long raphide and short bundle raphide found in tuber at three different harvest times. The largest single size was found in the tuber harvested before the plants shed their leaves, i.e., 229.67 ± 2.08 µm. The largest size of short bundle raphide was found in tuber harvested when the plants were shedding their leaves, i.e., 53.67 ± 3.51 µm.

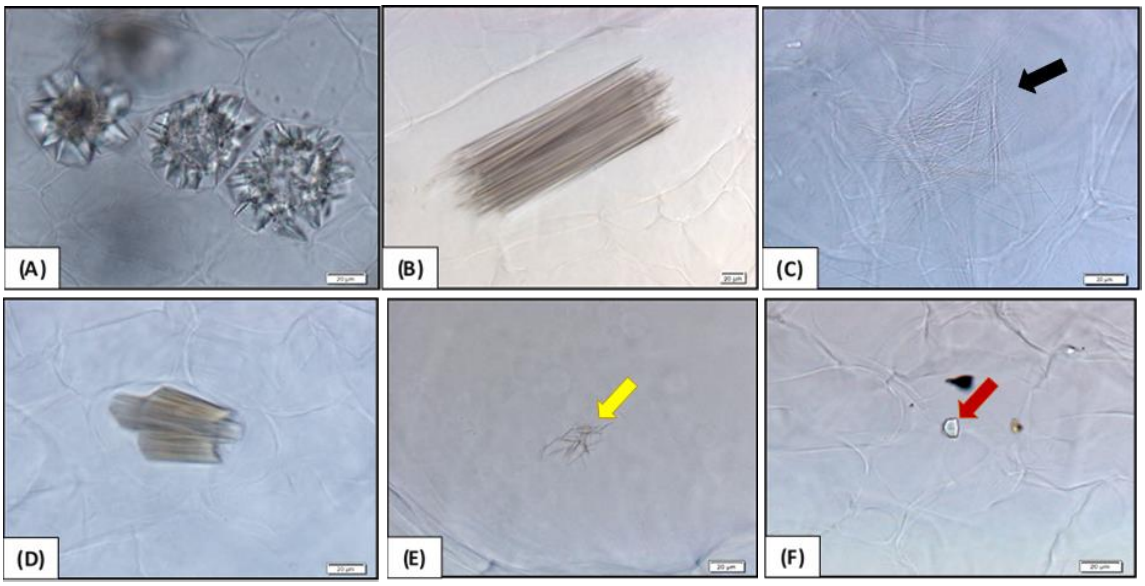

Figure 6. Calcium oxalate (CaOx) crystals found in porang tuber: (A) druse, (B) long organized raphide, (C) unorganized raphide (black arrow), (D) short organized raphide, (E) styloid (yellow arrow), (F) prism (red arrow).

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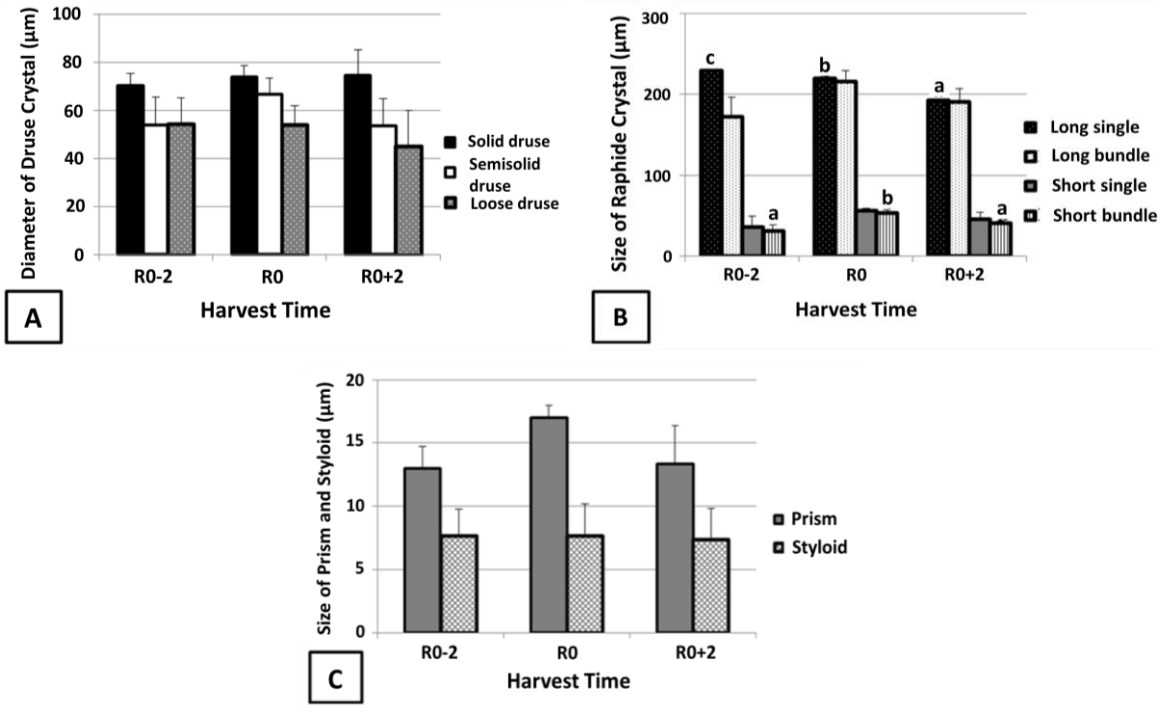

Figure 7. Size of each form of CaOx crystals in porang tuber in the third growing period: (A) druse, (B) raphide, (C) prism, and styloid. **Note:** Different letters (in one picture) in the same crystal form show significant differences based on the 0.05 Tukey Test; R_0-2: two weeks before the plants shed their leaves; R_0: when the plants were shedding their leaves; R_0 + 2: two weeks after the plants shed their leaves. The vertical bar shows SD (Standard Deviation) (n = 3)

The difference in crystal size between single long raphide and short bundle raphide in tuber harvested at three different harvest times was believed to be due to the influence of temporal regulation. There was no significant difference between crystal size variations of solid druse, semisolid druse, loose druse, long bundle raphide, short single raphide, prism, and styloid. However, the crystal size in the tuber tended to be different at three harvest times (Fig. 7). The crystal size of semisolid druse (Fig. 7A), long bundle raphide, short bundle raphide, short single raphide (Fig.7B), and prism (Fig. 7C) found in the tuber when the plants were shedding their leaves tended to be larger than the size before and after the plants shed their leaves.

However, it differed from the variation in the size of the solid druse crystals (Fig. 7A). The solid druse crystal size found in the tuber when the plants were shedding their leaves tended to be the same as it was at the time after the plants shed their leaves. It was also different from the size variation of loose druse crystals (Fig. 7A) and styloid (Fig. 7C). The loose druse and styloid crystal size found in the tuber when the plants were shedding their leaves tended to be the same as it was at the time before the plants shed their leaves. Similarly, the size variation of the single long raphide crystal (Fig. 7B), whose crystallite size in the tuber before the plants shed their leaves was greater than it was when the plants shed and after the plants shed their leaves.

The size of CaOx crystals is assumed to be influenced by time and the level of Ca. According to Mazen *et al.* [34], the size and the number of crystals in plants will increase in line with calcium levels in the planting medium. The morphology of CaOx crystals is very complex. Mutations affecting proteins, lipids, and polysaccharides contribute to changes in the size and shape of CaOx crystals [44]. This statement is supported by Franceschi and Nakata [18], who argue that variations in crystal size in a species can be influenced by intrinsic factors (genetic factors). For example, the function of cells in which crystals are formed and environmental factors, such as the amount of calcium available during the crystal formation process.

According to Nakata [45], an increase in the size of CaOx crystals plays a role in providing insect resistance. It can be argued that crystal size is one of the potential factors preventing the attack of herbivores on plants. Based on the research of Prychid *et al.* [46], various raphide idioblast sizes were found in *Amorphophallus*'s

leaves. However, idioblasts are getting more prominent as the plants mature. The research has also shown that the distribution of crystals is affected by organ maturity. Prychid *et al.* [46] also mentioned that the highest number and the largest size of druse crystals were found in mature *Amorphophallus* leaves. López-Macías *et al.* [47] also revealed that the size of CaOx crystals in mature plants tended to be greater than during the initial growth and development phase. According to Bernardino-Nicanor *et al.* [48], Ca increases along with the age of the plant, and the morphology and size of crystals can be affected by the growth stage. In addition, it has also been argued that calcium concentration and salinity in soil can influence the shape and distribution of CaOx crystals [49].

CONCLUSION

The tuber weight factor influenced the positive correlation between glucomannan content, calcium oxalate (CaOx) content, and crystal density. The result showed that 24.7% of crystal density and 39.2% of CaOx content was affected by glucomannan content. The increase in glucomannan could lead to increased calcium oxalate synthesis and the number of crystals. The highest total density of CaOx crystals in the porang tuber harvested when the plants were shed, i.e., 40,020 ± 16,554 crystals.cm^{-2}. An increase in CaOx content could increase the number of CaOx crystals. While an increase in CaOx crystals' density could increase the porang tuber's weight, different harvest times did not affect the size variation in some shapes of CaOx crystals. The single long raphide and short bundle raphide crystals showed different sizes at three harvest times, while the other shapes did not show differences in shape. These results indicate that the size of some CaOx crystals is affected by harvest time and other factors.

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