Research Article

The Dynamic of Calcium Oxalate (CaOx) in *Porang* Corms (*Amorphophallus muelleri* Blume) at Different Harvest Time

Nurul Chairiyah [1]*, Nunung Harijati [2], Retno Mastuti [2]

[1] Department of Agrotechnology, Faculty of Agriculture, University of Borneo, Tarakan, Indonesia
[2] Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia

ABSTRACT

The research aims to observe the influence of harvesting time on the change of calcium oxalate (CaOx) content and crystal density in *Porang* corms. The corms were harvested at different times, i.e., (1) two weeks before the plants shed (R0-1), (2) when the plants shed (R0), and (3) two weeks after the plants shed (R0+1). CaOx was obtained using the modified extracting method. Microscopic observations were obtained from the slices of the edge and *center* part of *porang* corms. Parameter observed including CaOx content, corm weight, shape, and density of CaOx crystal. CaOx content and crystal density in corms were analyzed using One way ANOVA. If the results are significant, it will be followed by Tukey Test α 0.05. In the meantime, the relation between CaOx content and corm weight was analyzed using Correlation Test Bivariate. The results showed that CaOx content was relatively higher in *porang* corms, i.e., $15.98 \pm 0.60g/100g$. On the other hand, the increasing of CaOx content might improve corm weight. The total density of druse, styloid, and prism crystal was pretty high in corms obtained when the plants shed compared to another harvest time, i.e., $1,494 \pm 286$; $31,280 \pm 17,406$ and $6,256 \pm 1,533$ crystals/cm^2. Raphide crystal density, by contrast, increased in corms obtained after the plants shed, i.e.,$1,656 \pm 368$ crystals/cm^2. Total CaOx crystal density in the edge parts of corms harvested when the plants shed was proportionately higher than in the other harvest times, i.e., $12,292 \pm 4,687.89$ crystals/cm^2. In contrast, CaOx crystal densities in the *center* parts of corms were not much different at three harvesting times. The density of druse and prism crystals was somewhat higher in the *center* part of corms than in the edge parts. In opposition to, the density of raphide and styloid crystals was fairly higher in the edge part of corms than it was in the *center* parts. However, only raphide crystal density found in the edge and *center* part of corms was significantly affected by harvest time from all these results.

Keywords: CaOx content, Crystal density, Corms, Different, Harvest time

Introduction

Porang corms have high economic value because they contain glucomannan, good for health [1 -5]. The corms are generally harvested in the 3rd growing period when the plants shed and after the plants [6 - 9]. It is also supported by the research of Chairiyah *et al.* [10] stated that glucomannan content in corms tended to be higher when the plant shed than it in another harvest time. The variation of glucomannan content due to metabolic differences is presumed to be ac-companied by differences in CaOx contained in the corms.

Oxalate compound, which is the raw material of calcium oxalate, has many benefits in plants, including calcium regulation. Maintaining ion balance plays a role in defense mechanisms for plant protection, tissue support, and heavy metal detoxification [11]. Oxalate compounds in plants can be found in the form of dissolved and not dissolved. Dissolved oxalate is usually formed

when oxalate compound bind with sodium (Na^+), potassium (K^+) and ammonium ($NH4^+$) ions. In contrast, undissolved forms of oxalate will form if oxalate compounds bind with calcium (Ca^{2+}) ions, magnesium (Mg^{2+}), and iron (Fe^{2+}) [12]. Oxalate content in plants commonly varies. The variation is influenced by several external and internal factors, i.e., (1) fertilizer use and soil chemical content, (2) climate factors, (3) genetic factors, and (4) other agronomic factors [13 - 25].

CaOx crystal is an ergastic material in plants. It is usually resulted from the binding of calcium (Ca) and oxalic acid ($C_2H_2O_4$) [26 - 32]. CaOx crystal formation needs 3-80% oxalate and 90% calcium (Ca) in plants [27]. The crystal could cause swelling and irritation in the mouth and throat if consumed [27, 32]. Several studies have also revealed that both oxalate compounds and calcium oxalate crystals can cause kidney disorders [33 - 36]. Although it is reported to have negative effects, CaOx crystals have a beneficial role for plants, including playing a role in defense mechanisms against herbivorous insects [27, 37]. Coté & Gibernau [38] also stated that CaOx crystals in some plants from the Family Araceae protect gametes and embryos from insect predation. Not only for a plant defense mechanism, these crystals also can diffract the sunlight to prevent the degradation of palisade chloroplast [39]. These crystals also play a role in the mechanism of excess calcium regulation in plants [40 - 45]. Other research also revealed that CaOx crystals have also functioned as an internal carbon source in plants if needed [46]. There are five basic CaOx crystals forms, i.e., druse, raphide, styloid, prism, and sands [27, 47]. However, CaOx crystal forms are found in *porang*, i.e., druse, raphide, styloid, and prism [48 - 50].

The period of *porang* corms development might affect CaOx content, the density of each CaOx crystal form, and its distribution in corms. Çaliskan [51], explained that oxalate content in plants varied based on the aging plant, time, weather, and soil type. In some plants, such as rhubarb, oxalate content instead increased in mature plants. The other plants, such as spinach, sugar beet, and banana, oxalate content instead increased during the early stage of development and decreased when the plants became mature. According to Indriyani [52], the growth period's CaOx crystal density in *porang* may be influenced. It is because oxalate content and crystal idioblast densities in *porang* corms varied at different growth periods. On the other hand, Liu *et al.* [53] stated that the difference in harvest time

might influence the accumulation of the chemical compound in konjac corms. This phenomenon may be occurred because of the difference in metabolisms. Physiological conditions in an organ are assumed to be different, so the metabolism results, in the case of CaOx, are thought to be unequal. Based on research from Nurlaila *et al.* [54] it is known that CaOx crystal density was tended to be higher in the *center* parts of *porang* corms than it was in the edge parts at different growing periods.

Conversely, based on observations from Chairiyah *et al.* [55], CaOx crystals were distributed almost equally on the edge and *center* parts of the corms in *porang* plants were exposed or not exposed to sunlight. Also, it is also known that the *walur* and *suweg*, which are taxonomically related to *porang*, have differences in CaOx crystal density at the edges and *center* parts of the corms [56, 57]. However, the physiological condition in *porang* corms at different harvest time is still clearly undetermined. Therefore, the dynamics of CaOx content, crystal density, and its distribution in *porang* corms need to be observed.

Material and Methods
Experimental Site

Porang corms were derived from Sumberbendo, Saradan Sub-district, Madiun East Java. The corms were obtained from the 2nd growth period; *porang* plants were planted to grow the 3rd growing period's vegetative phase. Nine corms with an average weight of 0.9-1.2 kg and a diameter of 15-16 cm. It was planted in a polybag that had compost as a planting medium. Each polybag was put with a distance of about 50 cm. Planting of corms was conducted until the late vegetative phase, i.e., the 6th month after it was planted. Afterward, harvest time was determined at three different times, i.e., two weeks before the plants shed (R0-1), when the plants shed (R0), and two weeks after the plants shed (R0+1).

Calcium Oxalate (CaOx) Content Extraction and Analysis

Determination of CaOx content applied Iwuoha & Kalu method [58], which had modified. This method consists of three stages, i.e., (1) digestion process, (2) oxalate precipitation, and (3) permanganate titration.

(1) Digestion process.

The cuts of wet *porang* were grated, and subsequently, the grating result was dissolved by aquadest to have a final concentration of 10%.

Afterward, the suspension was digested by adding 10 ml of HCl 6 N. It was heated at a temperature of 100°C for 1 hour, whereupon cooled. Later on, it was diluted by adding aquadest until the volume reached 250 ml. This process's last stage was filtering the suspension using Whatman filter paper (Grade 1: 11 µm).

(2) Oxalate precipitation.

In the oxalate precipitation process, 4 drops of methyl red were added to the filtrate. After that, a few drops of NH_4OH were added until the pH reached 4-4.5. Then the filtrate was heated at 90°C; then, it was cooled. Later on, it was filtered using the Whatman filter paper to remove deposits containing Fe (iron) ions. Afterward, the filtrate was reheated and added 10 ml of 5% $CaCl_2$, subsequently homogenized with a magnetic stirrer. After the homogenization process, the solution was centrifuged for 10 minutes at 2500 rpm. The supernatant was decanted, and the pellet was dissolved using 10 ml H_2SO_4 of 10 ml.

(3) Permanganate titration.

The pellet was dissolved with 10 ml of H_2SO_4 of 10 ml. Afterward, it was dissolved by adding aquadest until the volume reached 100 ml. Shortly after that, the solution was heated until it was almost boiled. Later on, it was titrated using 0.1N $KMnO_4$, which had been standardized to produce a light pink color for ± 1 minutes. CaOx content was calculated using the formula (1) [58]:

$$CaOx\ Content\ (g) = \frac{V \times M \times ME \times DF \times}{m_f \times 1000}$$

(1)

V : Volume of $KMnO_4$ (ml)
M : Molarity of $KMnO_4$
ME : Molar equivalent of $KMnO_4$ contained in oxalate
DF : Dilution factor
m_f : mass of wet *Porang* used

The water content obtained, later on, was used for the determination of corms dry weight. it was calculated using the formula (2) and (3) [10]:

$$WC\ (\%) = \frac{WW_1(g) - DW_1(g)}{WW_1} \times 100\%$$

(2)

WW_1 : Weight of grated fresh *porang* corms (weight before drying) (g)
DW_1 : The weight of grated corms which wasdried until it reached a constant weight (g)

WC : Water content contained in wet *porang* corms (%)

$$DW_2\ (g) = WW_2 - \left[\left(\frac{WC_2}{100\%}\right) \times WW_2\right]$$

(3)

WW_2 : Weight of grated fresh *porang* corms (weight before drying) (g)
WC : Water content contained in wet *porang* corms (%)
DW_2 : The weight of grated corms which was dried until it reached a constant weight (g)

The dry weight corms were subsequently used to determine calcium oxalate (CaOx) content. It was calculated using the formula (4) [59]:

$$CaOx\ content\ (^g/_g) = \frac{C}{DW_2}$$

(4)

C : CaOx mass (g)
DW_2 : The weight of grated corms, which was dried until it reached a constant weight (g)

Preparation of Microscopic Slide

Samples for microscopic slides were derived from the edge and *center* part of the corms' tissue slices. The making of semi-permanent slides used the modified clearing method of Ilarslan *et al*. [60]. Each part (the edge and the *center* part) of the corms had three slides to be observed. The organs were sliced using a sliding microtome with thickness ± 10 µm. The tissue slices were soaked in NaOH 5% for ±24 hours at 37°C.

Furthermore, the tissue slices were soaked in commercial sodium hypochlorite solution 50% for one hour to clear the tissue, and then they were rinsed under running water (or with plenty of water). Furthermore, they were soaked with various ethanol concentrations, starting from 30%, 50%, 70%, 80%, for 10 minutes each and 100%EtOH, for 5 minutes. After that, the tissue slices were placed on the object glasses spilled with hoyer and covered with the cover glasses.

Microscopic Observations

Microscopic slides were observed with a binocular light microscope (Olympus CX31 type; Japan). Variety of CaOx crystal was observed at 100× - 1000× magnification, whereas the density of CaOx crystal was counted at 100 × and 1000× magnification. CaOx crystals observed at 1000× magnification was grouped into small crystals,

whereas CaOx crystals observed at 100 × magnification was grouped into a giant crystal. The number of CaOx crystals was observed on three microscope fields of view from each microscopic slide using a hand tally counter. It eventually was calculated to determine the crystal density. It was calculated using the formula below (5) [55]:

The total density of CaOx crystals per slide (S$_1$)=	$\dfrac{(\Sigma \text{ total CaOx crystals}) / 3}{\text{Large field of view (cm}^2)}$
The total density of CaOx crystals per replicates (R)= (S$_1$ + S$_2$ + ...+S$_n$)/n n= number of slides were made per replicate	
The total density of CaOx crystals per harvest time (T)= (R$_1$ + R$_2$ + ...+R$_n$)/n n= number of replicate were made per harvest time	

(5)

A large microscope field of view was calculated bymeasuring off the microscope field of view using an ocular and objective micrometer. The diameter was included in the circle area formula to obtain an area of fields of view of the microscope. The unit area of the field of view of the microscope was converted from µm^2 to cm^2. CaOx crystals found in microscopy slides were documented using a digital camera (Canon IXUS 120 IS type; Japan).

Data Analysis

This research used independent variables, eq harvest time, dry weight corms edge and *center* part of corms. Meanwhile, the dependent variables were CaOx content and the number of crystals per unit area. Data of CaOx content was obtained from the extraction of *porang* corms at different times, repeated three times. At the same time, the data of CaOx crystal density was calculated from the average number of crystals found in the edge and *center* part of the corms. Every harvest time had three corms as replicates. The data were tested by the One Way ANOVA Test using software SPSS Statistics 17.0. It was conducted to analyze the influence of harvest time on CaOx content and crystal density. It was conducted to determine whether harvest time can influence CaOx content and crystal density in *porang* corms. The result of ANOVA Test will be tested by Tukey Test α 0,05 in case it was significant. A bivariate correlation test was performed to determine the CaOx content of the relationship to the dry weight of *porang* corms.

Correlation assessment between CaOx content and weight of *porang* corms was analyzed based on a range of numbers -1 to +1 and a range
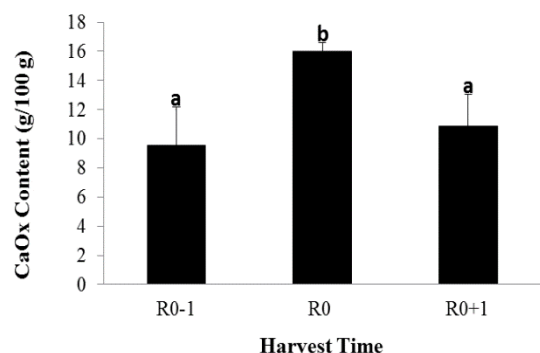

Figure 1. CaOx content at three harvest times in *porang* corms (*A. muelleri*) in the third growth period. Note: Different letters (in one picture) showed significant differences based on The Tukey Test α 0.05 (1). R0-1: two weeks before the plants shed (2). R0: When the plants shed (3). R0 + 1: two weeks after the plants shed. The vertical bar showed SD (Standard Deviation) (n = 3)

of numbers from 0 to 1. The sign (+) shows the directional relationship between variables x and y. The greater the variable x, then the more significant the variable y. In contrast, the character (-) shows the opposite relationship between variables x and y. Number 0 to 1 indicates the existence of correlation. The number 0 means there is no relationship between the variables x and y. On the contrary, correlation number 1 shows the perfect relationship between variables x and y (very strong correlation) [61].

Results and Discussions
The Dynamics of Oxalate Content at Three Harvest Times in Porang Corms (A. muelleri)

Based on the results, there was a significant difference between the CaOx content at three harvest time. It was showed by a significance value that was smaller than α value (0.05). *Porang* corms were harvested when the plants shed had the highest content of CaOx, ie 15.98 ± 0.60 g/100 g of dry weight corms (Figure 1). The difference in CaOx content allegedly caused by differences in the metabolism of the corms at three harvest times.

Based on previous research, the oxalate content, which was a compound forming CaOx, in plants could vary based on certain factors: harvest time, growth stage, and harvesting interval [11, 14, 23, 51, 62 - 66]. Middleton & Barry [67] also explained that the CaOx content in grass plants during the early stages of growth had an
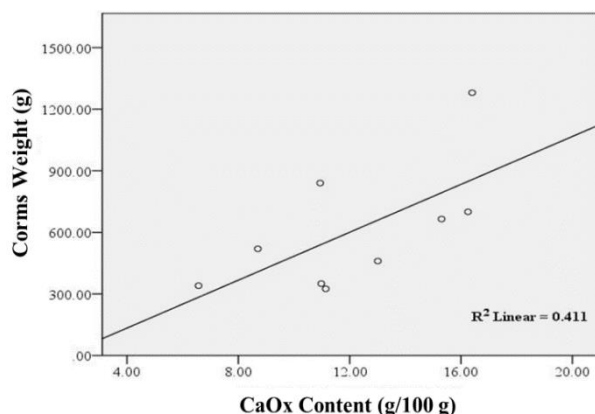
Figure 2. The Relationship between calcium oxalate (CaOx) content and weight of *porang* corms (*A. muelleri*) in third growth period
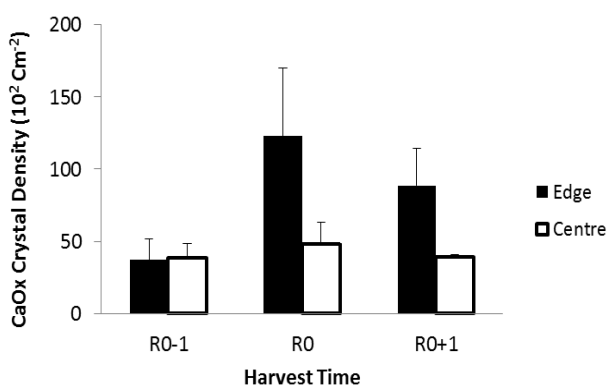

Figure 3. CaOx crystal density in the edge and *center porang* corms (*A. muelleri* Bl.). R0-1: two weeks before the plants shed; R0: when the plants shed; R0+1: two weeks after the plants shed.

oxalate content that tended to be higher than it in the next growth stage. The next growth stage showed that the CaOx content in each grass plant was tended to experience a decline, especially in the third growth stage.

The high content of CaOx in corms was harvested when the plants shed (R0), presumably influenced by the increase of plant metabolic activity. When the plants matured, glyoxylate synthesis activity, which was one of oxalate forming precursors, allegedly increased. Therefore, it could be said that the oxalate content in adult plants also increased. There was an assumption that oxalate produced from the synthesis eventually accumulated in a reasonably high content in several plant organs, one of them is corms. It is supported by McGoodwin [68] who stated that photosynthetic activity had increased in mature plant leaves. Increasing of photosynthetic activity was assumed to rise photorespiratory activity. It

was presumed to increase the glyoxylate, which is one of the oxalate precursors. This assumption was supported by Khan [69], Kisaki & Tolbert [70], and Lindqvist & Brändén [71]. Their studies explained that the glycolic oxidation process produced glyoxylate by using glycolic oxidase enzyme during photorespiration. Libert & Franceschi [11] also stated that glycolic was produced in chloroplasts during the photorespiration process; later on it was converted to glyoxylates in peroxisomes. The glyoxylate resulted was a primary precursor of oxalate formation in plants. This statement is also supported by Burrows & Tyrl [72], who stated that oxalate concentration increased along with plant maturity. Oxalate concentration was highest when plants get old and dry out. It was presumed that other factors play a role in increasing CaOx content, i.e. absorption rate and calcium accumulation [27, 73, 74].

Increasing metabolism that affected the rising of corms weight due to rising food reserves production [75]. It also influences the increase of oxalate production, a compound forming CaOx [28, 69 - 71]. Although both can be influenced by metabolic activity, the corms weight and calcium oxalate content were not particularly related in this research. Based on the results of the correlation test, it was known that the CaOx content was positively correlated with corms weight. This correlation was strong because it was greater than 0.5, i.e., 0.636. However, because the significance value was greater than α (0.05), i.e., 0.066 or the determination value was 41% ($R^2 = 0.411$) (Figure 2), so that it could be said that 41% of corms weight variation was influenced by CaOx content and the rest 59% were influenced by other factors, e.q. glucomannan, water content, and carbohydrates [75].

CaOx Crystal Density in the Edge and Center Parts of Porang Corms (A. muelleri Bl.) at the Different Harvest Times

There was no significant difference in total CaOx crystal density in the edge and *center* parts of corms at the different harvest times (Figure 3). However, there was a possibility of temporal regulation for CaOx crystal density. It was shown through the highest and lowest total CaOx crystal density in every part of the corm. The lowest total CaOx crystal density was found in the edge of *porang* corms harvested at two weeks before the plants shed, i.e., $3,726 \pm 1,422.60$ crystals/cm^2. The highest total CaOx crystal density was found in the edge of *porang* corms harvested when the plants were shed. The thickness was four times
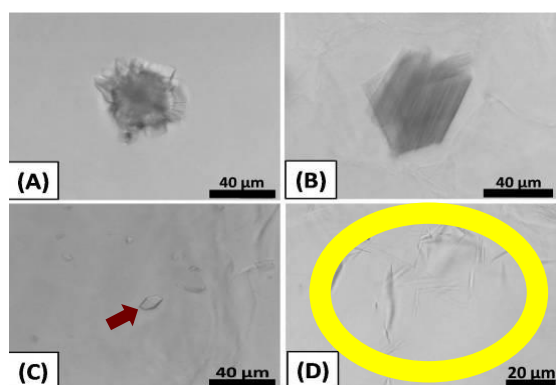
Figure 4. CaOx crystals that found in *porang* corms (*A. muelleri* Bl.) from the third growing period: (A) druse crystal; (B) short raphide crystal; (C) prism crystal (red arrow); (D) styloid crystal (yellow circle).
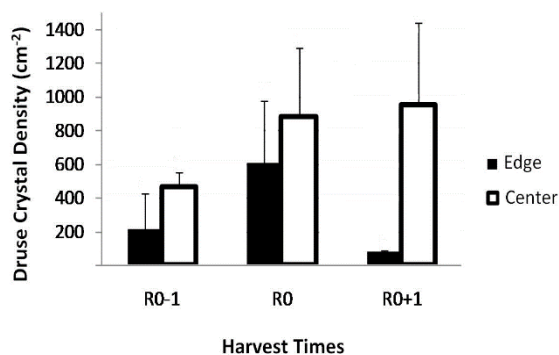

Figure 5. Druse crystals density in the edge and *center* part of *porang* corms (*A. muelleri* Bl.). R0-1: two weeks before the plants shed; R0: when the plants shed; R0+1: two weeks after the plants shed.

more than the lowest one, i.e., $12,292 \pm 4,687.89$ crystals/cm^2. CaOx crystal density in the edge of *porang* corms tended to decline at two weeks after plants the shed, i.e., $8,854 \pm 2,608.91$ crystals/cm^2.

CaOx crystal density in the *center* parts of corms was not too different. *Porang* corms were harvested at different time tended to have similar crystal density, i.e., $3,867 \pm 981.75$; $4,818 \pm 1,486.35$; and $3,889 \pm 184.94$ crystals/cm^2. The accumulations of crystal density were almost the same in the three different harvest times, indicating that the temporal regulation did not overly influence the corms' center part.

Although crystal density in the edge and *center* parts of corms were not significantly different, crystal density in the edge part of corms tended to be higher than it did in the *center* part.

It assumed CaOx crystal played a role in the mechanism of protection against pests. This assumption was supported by Brubaker and Horner [76], which explained that CaOx crystals were often formed in the epidermal and subepidermal tissues. The distribution of CaOx crystals in the tissues played a role in structural reinforcement on the tissue protector [77].

The Density of Each CaOx Crystal Form in the Edge and Center Part of Porang Corms (A. muelleri Bl.)

Based on observations, there were no differences in CaOx crystal forms on edge and in the *center* parts of corms from the third growing period. The crystals were found at the time of observation of the edge and *center* parts of the corms, i.e., druse, raphide, prism, and styloid (Figure 4). Each CaOx crystal form tended to have different densities in the other parts of the corms and at different harvesting times.

Druse crystals density found in the edge and *center* parts of corms in the three harvest time tended to be different (Figure 5). Druse crystal density in the *center* parts of corms tended to be higher than in the edge parts. The highest druse crystals density was found in the *center* parts of corms harvested at two weeks after the plants shed, i.e., 952 ± 490.19 crystals/cm^2. The lowest druse crystals density was found in the edge parts of corms harvested at two weeks after the plants shed, i.e., 82 ± 8.95 crystals/cm^2. Druse crystal density in the *center* parts of corms, which was higher, was assumed that related to the role of CaOx crystal as reinforcement structural of tissue.

On the other hand, prism crystals density in the *center* parts of corms also tended to be higher than it did in the edge parts (Figure 6). The highest prism crystals density was found in the *center* parts of corms harvested when the plants shed, i.e., $3,928 \pm 1,008.05$ crystals/cm^2. The lowest prism crystals density was found in the edge parts of corms harvested at two weeks before the plants shed, i.e., $1,455 \pm 1,049.22$ crystals/cm^2.

Druse and prism crystals density tended to be higher in the *center* parts of corms because it might be related to CaOx crystal's role for structural reinforcement on the tissues protector. It could be proven through the structure of the *center* part of corms used in the study tended to be more challenging and denser than it did in the edge parts. According to Webb [31], CaOx crystals could serve as structural reinforcement coincides with cell wall sclerification, e.g., crystals
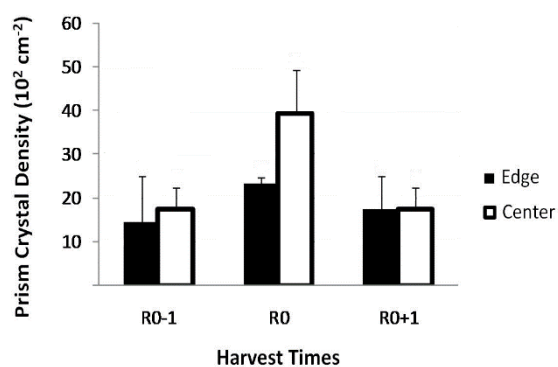
Figure 6. Prism crystals density in the edge and *center* part of *porang* corms (*A. muelleri* Bl.). R0-1: two weeks before the plants shed; R0: when the plants shed; R0+1: two weeks after the plants shed.

Figure 7. Raphide crystals density in the edge and *center* part of *porang* corms (*A. muelleri* Bl.). R0-1: two weeks before the plants shed; R0: when the plants shed; R0+1: two weeks after the plants shed.

Figure 8. Styloid crystals density in the edge and *center* part of *porang* corms (*A. muelleri* Bl.). R0-1: two weeks before the plants shed; R0: when the plants shed; R0+1: two weeks after the plants shed.
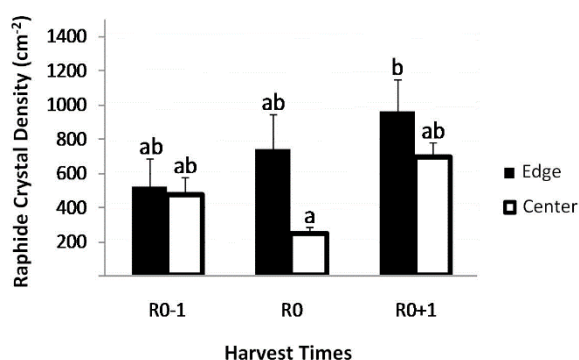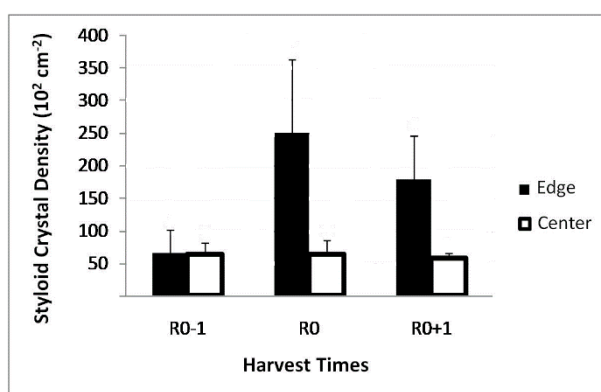
found in epidermal and subepidermal tissues [76].

Rafida crystals found in the edge and *center* parts of corms at three different harvest times have different densities (Figure 7). Unlike druse and prism crystals densities, which were relatively high in the *center* parts, raphide crystals density was relatively high in the edge parts of corms. The highest raphide crystals density was found in the edge parts of corms harvested two weeks after the plants shed. It reached 959 ± 192.40 crystals/cm^2. The lowest raphide crystals density was found in the *center* parts of corms harvested when the plants shed, i.e., 248 ± 38.95 crystals/cm^2. Raphide crystals density in the edge parts of corms was assumed to be related to CaOx crystals' role as a defense mechanism of pests and herbivorous animals.

Like raphide crystals, styloid crystals density found in the edge parts of corms tended to be higher than it in the *center* parts (Figure 8). The highest styloid crystals density was found in the edge parts of corms that were harvested when the plants shed, i.e., $25,024 \pm 11,225.44$ crystals/cm^2. The lowest styloid crystal density was found in the *center* parts of corms gathered at two weeks after the plants shed, i.e., $5,819 \pm 769.60$ crystals/cm^2. Styloid crystals density in the edge parts of corms, which was higher, was guessed related to CaOx crystals' role as a defense mechanism of pests and herbivores.

Based on the research from Sakai *et al.* [78] and Thurston [79], acicular CaOx crystals, like raphide and styloid, were often formed in the particular cell that also could produce toxin compounds. These crystals also facilitated the spreading of toxins through the herbivore skin. According to Sakai *et al.* [78], ingestion of plant tissues that contained raphide crystals usually could irritate the herbivore's mouth and throat. This irritation could occur in two ways, i.e., (1) mechanical irritation by CaOx crystal, like raphide crystal, and (2) chemically irritation by toxin compounds in the crystals.

CaOx Crystal Form Density of Porang Corms in Different Harvest Time

There was no significant difference of each CaOx crystal density at three different harvest times. It was assumed harvest time interval was too short, i.e., only two weeks, so the CaOx crystal formation level was not extremely different. Despite the fact that it did not have a significant difference, each crystal's density tended to be higher in corms was obtained when the plants
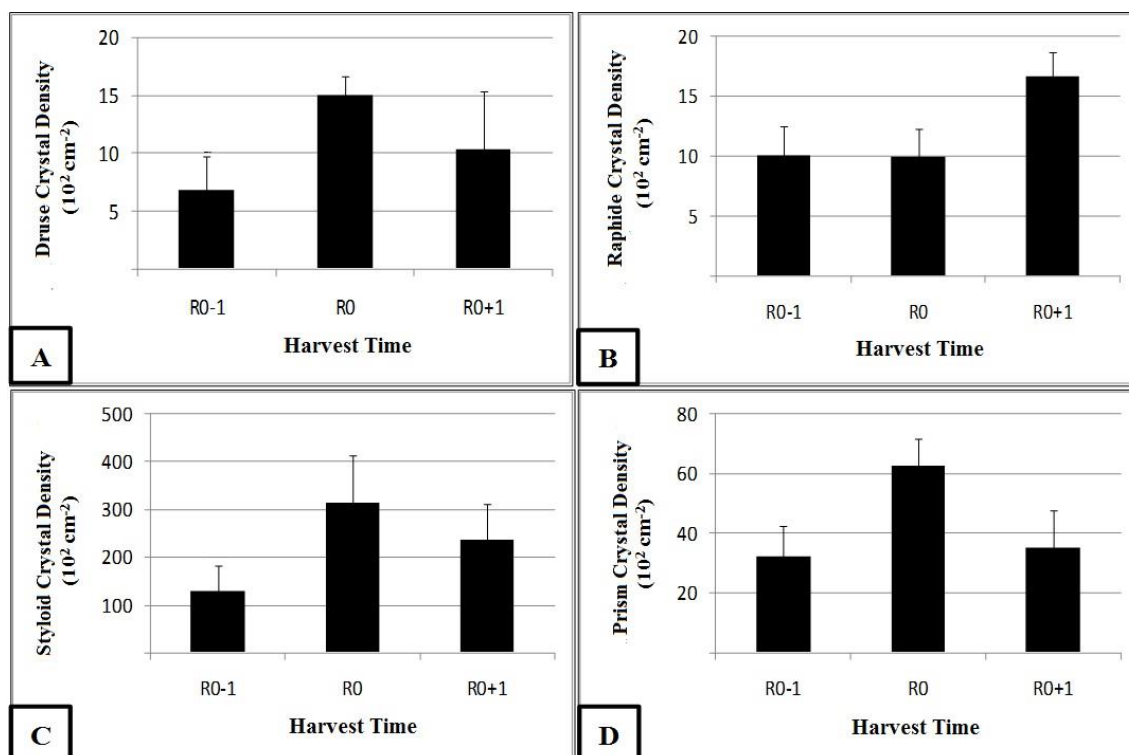
Figure 9. The density of each CaOx crystal form in *porang* corms (*A. muelleri*): (A) druse crystal density, (B) raphide crystal density, (C) styloid crystal density, (D) prism crystal density.
R0-1: two weeks before the plants shed; R0: when the plants shed; R0+1: two weeks after the plants shed. Vertical bar showed SD (*Standard Deviation*) (n= 3).

shed than it did in another harvest time. The density of druse, styloid, and prism crystals was found in corms was obtained when the plant shed, i.e., $1,494 \pm 286$; $31,280 \pm 17,406$; and $6,256 \pm 1,533$ crystals/cm^2. These crystals' density tended to be decreased in *porang* corms obtained after the plant's shed or dormancy period (Figure 9 A, C, D). In contrast, density of raphide crystal was found in observation tended to be higher in corms were obtained after plant shed than it did in another harvest time, i.e., $1,656 \pm 368$ crystal/cm^2 (Figure 9 B).

Decreasing of druse, styloid, and prism density in *porang* corms was obtained after the plants' shed is assumed to decrease metabolism activity and oxalate compound oxidation. Moreover, herbivorous insects and fungi lead to raphide crystal formation in a plant as one of the defense mechanisms' responses. The density of druse, prism, and styloid density in corms were obtained when the plants shed might be related in harvest time or the intervals. This assumption was supported by Burrows & Tyril [72], which explained that oxalate concentration would increase along with plant maturity and development.

McGoodwin [68] explained that photosynthe-sis activity would increase in the mature plant leaf and supports the assumption of plant age influence towards CaOx crystal formation. Increasing photosynthesis activity is assumed to increase photorespiration activity. Increasing photorespiration activity will raise glyoxylate synthesis. Glyoxylate is known as one of the precursors of oxalate formation [11, 69 - 71]. If this oxalate bound Ca was obtained from the environment, it would be formed precipitation in particular cell, namely idioblast. The precipitation is in the form of CaOx crystal.

Increasing raphide crystal density in corms after plant shed might be related to defense mechanism towards the attack of herbivorous insect and fungus. Raphide crystals play a role in plant defense mechanisms against herbivorous animals [27, 80-84]. Molano-Flores [83] proved that increasing crystal density in plants could be affected by herbivory. The presence of raphide crystal in a plant is commonly accompanied by cysteine protease. Collaboration raphide crystal and cysteine protease affected growth and cause mortality of caterpillar. The needle effect of raphide crystal can particularly improve bioactive factors by damaging cell membrane, cuticle, epi-

thelium, the nuclear membrane, etc. This effect has a function to enable bioactive factors to penetrate the membrane and stimulate allergy reactions [37].

Conclusion

Harvest time can affect the changing of calcium oxalate content in *porang* corms with the highest content found in corms at the plant sheds, i.e., 15.98 ± 0.60 g / 100 g. Among the density of four crystal forms found in the corms, only raphide crystal density in the edge and *center* part of corms was significantly affected by the harvest time. The highest density of raphide crystals was found on the edge of the corms harvested two weeks after the plant shed, i.e., 959 ± 192.40 crystals / cm^2.

Acknowledgments

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