

# Variation of Calcium Oxalate (CaOx) Crystals in Porang (*Amorphophallus muelleri* Blume)

Nurul Chairiyah\*, Nunung Harijati, Retno Mastuti

Biology Department, Faculty of Mathematic and Natural Sciences, Brawijaya University, Malang, Indonesia.  
Email: \*nchairiyah@gmail.com

Received June 25<sup>th</sup>, 2013; revised July 25<sup>th</sup>, 2013; accepted August 15<sup>th</sup>, 2013

Copyright © 2013 Nurul Chairiyah *et al.* This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

This research had aimed to determine variations in form of calcium oxalate (CaOx) crystals in porang. Slides for microscopic observation were prepared from sliced leaf, petiole, and tuber of porang. Sliced organs were cleared by using modified clearing method. The parameters that were observed included form and variety of morphology type of CaOx crystals. Microscopic observation showed there were four basic forms of crystals, *i.e.*, druse, raphide, prism and styloid with some variation of diverse types, each of which had 49, 10, 8, and 5 variations. CaOx crystals, based on the size, were grouped into large (20 - 250  $\mu\text{m}$ ) and small (1 - 15  $\mu\text{m}$ ) crystals.

**Keywords:** Porang; Variation; Shape; CaOx Crystals

## 1. Introduction

Calcium oxalate (CaOx) crystals can be found in all photosynthetic organisms including algae. The production of CaOx crystal in plants is a normal physiological process and as a potential defense mechanism. These crystals can be found in the vegetative, reproductive, storage, and developing organs, and also in photosynthetic and non-photosynthetic tissues [1,2].

CaOx crystals are formed from calcium (Ca) that derived from the environment and oxalic acid (2-carbon dicarboxylic acid). CaOx crystals' formation through several metabolic processes from different biochemical pathways, *i.e.* glyoxylate and ascorbic acid pathways [3]. According to Franceschi and Nakata [2], crystal formation required coordination of several different mechanisms. These mechanisms include expense of Ca from the apoplast. Ca transfer from the cytosol into the vacuole, and then it was transferred into the crystal chamber. Simultaneously, the oxalic acid was synthesized in the cytoplasm and transferred to the vacuole and the chamber.

CaOx crystal forms which were often found in plants were raphide (needle-shaped), prism, druse, styloid and sand crystals [1,2]. Prism crystal shaped like a rhombohedral box, which was generally found in the single form crystals per cell or more. Raphide crystal was a rectangular styloid crystal which had been thickened and lengthened. It could be found in the form of single crystal or

bundle crystal per cell in plant. Sand crystal was derived from an association of a small angular crystal. Druse crystals were derived from the combination of multifacet crystals. Druse crystal could consist of one or more per cell [2]. Styloid or pseudo-raphide crystal had a shape thicker than common raphide crystal and is usually solitary in the cells [4]. CaOx crystals which were found in the cell walls were generally rhombohedral or prismatic crystals, whereas crystals in the cells could be raphide, druse, and sands crystals [2].

Each form of CaOx crystals has varieties of morphological crystal types. This statement was supported by the researches of Keating [5], Cote [6], and Crowther [7] by using Araceae Family. They stated that raphide and druse crystals had varieties of morphology types, such as simple raphide, elongated raphide, druse, very small druse, biforine, and varieties of ends raphide crystals. Variations in the type of crystal morphology could be found throughout the plant organs.

Porang (*Amorphophallus muelleri* Blume) is a bulbous plant, which is native to tropical areas. Porang can cause irritation and itchy when it consumed because it contains calcium oxalate crystals [8]. Based on the results of research conducted by Prychid *et al.* [9], plant of the genus *Amorphophallus*, which was a member of Family Araceae, accumulated CaOx crystals, generally in raphide and druse crystal form. However, it was possible to find other

crystal types in *Amorphophallus* because some species which were grouped in Family Araceae could accumulate other crystal types in their tissue [1,6,10-14]. Therefore, it was interesting to observe the possibility of a variety of shapes and types of CaOx crystals in porang (*Amorphophallus muelleri* Blume). In different organs thought to have a variation of different forms of CaOx crystals, observations of a number of variations type of CaOx crystals in the porang leaves, petiole, and tuber were apparently valuable.

## 2. Materials and Methods

### 2.1. Preparation Microscopy Material

Leaf, petiole and tuber of Porang were used as microscopy material. The materials were harvested from eight weeks old of Porang

### 2.2. Preparation of Microscopy Slide

Preparation of microscopy slide from leaves sample were conducted by cutting  $1 \times 1 \text{ cm}^2$  at three locations per intact leave using razor blade. Different with leaves, petiole samples were obtained from skin and central part of petiole. Petiole sampling included top-, middle- and base-part of petiole, whereas tuber sampling taken from the edge and center of the tuber. Sample slices of petiole and tuber were prepared using hand clamp sliding microtome.

The cut and sliced sample was cleared according to Ilarslan *et al.* [15] with some modification as followed: all samples were soaked in 5% NaOH at  $37^\circ\text{C}$  for 24 hours. After incubation, the sample transferred to 50% sodium hypochlorite commercial at room temperature for 1 hour. Rinse in running tap water was required after soaked in sodium hypochlorite and followed by dehydration in alcohol series 30%, 50%, 70%, 80% for 10 minute to each sample. The last step of dehydration was conducted in 100% EtOH for 5 minutes. The cut or sliced object were place on slide glass, mounted with Hoyer solution and covered using cover glass. Now all slide ready to observe under light microscope (Olympus CX31) at 100, 400, and 1000 times magnification. The observed objects were documented using a digital camera 7.2 MP.

## 3. Results and Discussion

### 3.1. The Forms and Types of CaOx Crystals in Leaf, Petiole, and Tuber of Porang (*Amorphophallus muelleri* Blume)

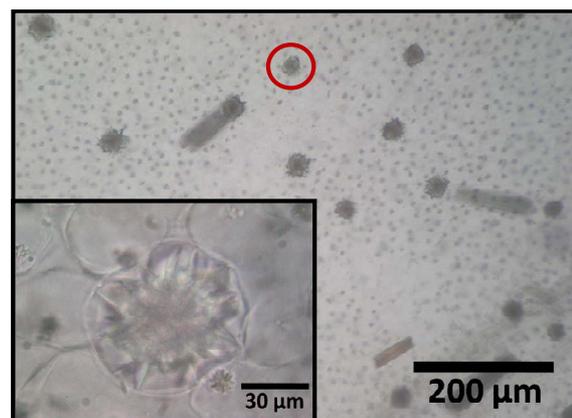
In general we found four basic forms of CaOx crystals in Porang (*A. muelleri*). They were 1) druse (large and small size), 2) raphide (bundle and single), 3) prism, and 4) styloid crystals. These results were different with Prychid *et*

*al.* [9] which stated *Amorphophallus* plants only accumulated raphide and druse crystals. However, Cote [6] found three basic forms of CaOx crystal in *Dieffenbachia seguine*, species that have same family with *A. muelleri*, Araceae family. The crystals included druses, raphides, and prisms. In detail the CaOx crystals that we found as below.

### 3.2. Druse Crystal

Druse crystals had a variety of sizes, types, globular forms and its constituent units. Such crystals were grouped into two categories, namely small druse which had 5 - 13  $\mu\text{m}$  in diameter and large druse with 20 - 135  $\mu\text{m}$  in diameter (**Figure 1**). Based on druse diversity type, we found four types, *i.e.* solid druse, semisolid druse, and loosely druse (**Table 1**).

The classification three types of druse crystal were based on differences in the composition of globular crystal that made up those crystals. We found druse crystal which had morphology thick ring in the edge and empty in center under observation at  $100\times$  magnification. However, when we altered focus by move up and down of microscope knob, we found center part actually not abso-



**Figure 1.** The difference between large and small CaOx crystals in Porang (red circle): Inset shows the difference between large and small druse crystal in leaf.

**Table 1.** Crystal druse types in porang (*A. muelleri*).

Type	Diameter ( $\mu\text{m}$ )	Globular Forms	Constituent Unit
Solid Druse		Densely	Like Rose Petals
Semisolid Druse	20 - 135	Only on the Edges	Like Rose Petals
Loosely Druse		There Is a Distance/Tenuous	Like Glass Flakes Prism
Small Druse	5 - 13	Densely	Stiloid Prism and Stiloid

lutely empty, it had very thin structure. We named semi-solid for such entire feature (**Figure 2(d)**). We called solid when in the center of crystal full with constituent of crystal and observation in detail showed that look like roses. The entire morphology of that crystal formed a perfect sphere and no concave on its structure (**Figure 2(c)**). The loose type was given to druse crystal which had less perfect of globular structure, in between constituent unit had tiny distance (**Figure 2(e)**). Distribution of big size crystal include leaves which had size 20 - 60  $\mu\text{m}$ , petiol and tuber with size 20 - 40  $\mu\text{m}$  and 30 - 135  $\mu\text{m}$  respectively. Apparently those variation sizes were caused by genetic variation.

Small druse crystals had diameter between 5 - 13  $\mu\text{m}$ , only could be observed under microscope with 1000  $\times$  magnification. These small druse crystals were grouped into three types based on the composition of crystals constituent. Small druse crystals were composed of styloid crystals only (**Figure 2(b)**), prism crystals only (**Figure 2(a)**), and mixed prism and styloid crystals. These small druse crystals had a transparent white color.

In leaf, druse crystals commonly were found in the adaxial site, which was the most frequently exposed to sunlight (**Figure 1**). Solid druse crystals were abundant in the leaf. Solid druse crystals generally were found in pairs with another solid druse crystal. Semisolid (**Figure 2(d)**) and loose (**Figure 2(e)**) druse crystals were rarely found in leaf. Small druse crystals that were found in leaf had several different forms, namely small druse crystals were composed of styloid crystals only (**Figure 2(b)**), prism crystals only (**Figure 2(a)**), and combination of prism and styloid crystals.

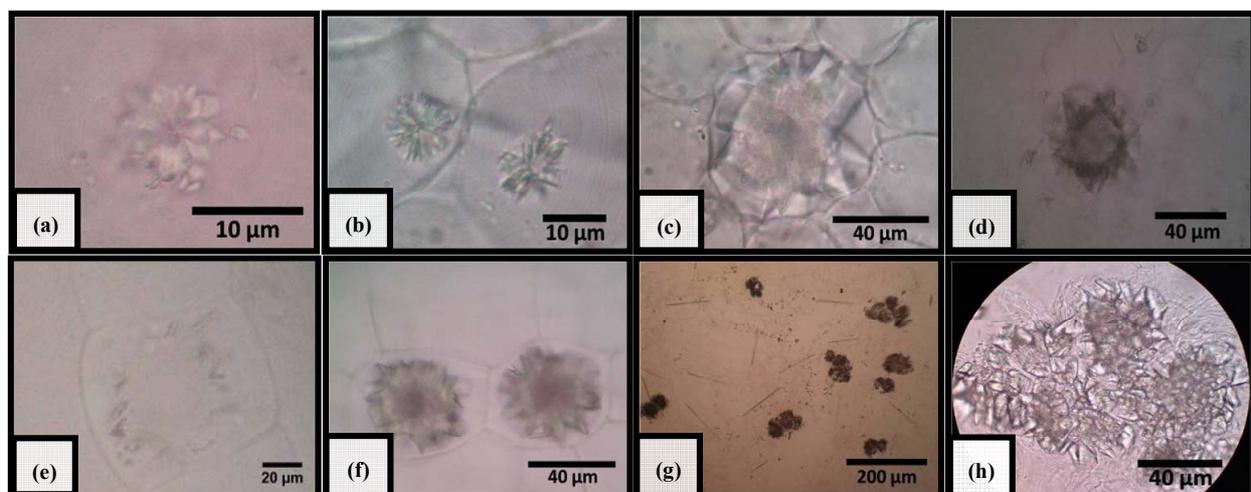
In petiole, druse crystals were found align vertically and sometimes in pair form. The types of druse crystal

which were found in petiole were solid druse, semisolid druse, and loose druse. Small druses were not found in this organ. Just the same as druse crystals which were found in the leaves, druse crystals which were found in petiole were located very close. The close form was two or three crystals coincide, or stuck together.

Druse crystal types which were found in tuber *i.e.* semisolid and loose druse crystals. As in the leaf and petiole, druse crystals were also found in pairs but not as much as in the leaf. And the crystals, especially the solid one had darker color than it found in the leaf. Generally druse crystals had form like roses. However, druse crystal in tuber had forms like a solid lump of noodles if observed under microscope with low magnification (**Figure 2(g)**). However, if it observed under the highest magnification, the forms of lump noodles were compact rather than scarce. The group of lump noodle form consisted of 2 - 5 druse crystals that were closely (**Figure 2(h)**). Noodless group crystals had a diameter 80 - 135  $\mu\text{m}$  in size. Some varies types of druse crystal that we found in line with Prychid *et al.* [9] statements. They mentioned, druse crystals were found in all of *Amorphophallus* species have variation of crystal number and size, ranging from one or several small crystals. Each druse was a group of various forms of inter-crystalline closed together each other.

### 3.3. Raphide Crystal

In addition to having variations in size and type, raphide crystals that were found in porang also had color variation (**Table 2**). Raphide crystals were found in the form of a single and bundle crystal. The size of crystal length grouped into long and short size. The long one had ranged from



**Figure 2.** Variation of druse crystal types in Porang: (a) Small druse crystal was composed of prism crystals; (b) Small druse crystal were composed of styloid crystals; (c) Solid druse crystal; (d) Semisolid druse crystal; (e) Loose druse crystal; (f) Solid druse crystals that coincide; (g) Noodles solid druse crystals; (h) Noodles druse crystal was composed of five solid druse crystals.

135 - 250  $\mu\text{m}$  in length and 37 - 80  $\mu\text{m}$  in length for short size. The classification was based on differences in shape, color, size, neatness of crystal edge, whole crystalline organization.

Bundle straight edge raphide crystals which had size 37 - 65  $\mu\text{m}$  in length was composed of a group of needle shape raphide crystals, well-organized and had straight edge. Bundle raphide which had size 37 - 65  $\mu\text{m}$  in length could be classified into short raphide crystals. This was because the length of the single raphide crystals that

made up the bundles tends to be shorter than the long one. Not straight edges short raphide crystal was composed of a group of short raphide crystal, well-organized but didn't have straight edge. Not neatly organized short raphide crystal was composed of a group of short raphide crystals and the arrangement of the crystals was not irregular but still organized. Not organized short raphide crystal was composed of a group of unorganized short raphide crystals and it was not irregular but still formed a group. Organized bundle short raphide crystal and each

**Table 2. Types of crystals raphide in porang (*A. muelleri*).**

Type	Colour	Size ( $\mu\text{m}$ )	Neatness of Crystal Edges	Whole Crystalline Organization		Additional Information	
				Organized	neatness		
Bundle	Black	135 - 250	straight	O <sup>a</sup>	N <sup>c</sup>	-*	
			Not straight	O <sup>a</sup>	N <sup>c</sup>	-*	
			Not straight	O <sup>a</sup>	nN <sup>d</sup>	-*	
			Not straight	O <sup>a</sup>	nN <sup>d</sup>	Puncture each other	
			Not straight	nO <sup>b</sup>	nN <sup>d</sup>	-*	
		straight	O <sup>a</sup>	N <sup>c</sup>	-*		
		Not straight	O <sup>a</sup>	N <sup>c</sup>	-*		
		37 - 80	Not straight	O <sup>a</sup>	nN <sup>d</sup>	-*	
		Not straight	O <sup>a</sup>	nN <sup>d</sup>	Puncture each other		
		Not straight	nO <sup>b</sup>	nN <sup>d</sup>	-*		
	Brown	37 - 80	straight	O <sup>a</sup>	N <sup>c</sup>	-*	
			Not straight	O <sup>a</sup>	N <sup>c</sup>	-*	
			Not straight	O <sup>a</sup>	nN <sup>d</sup>	-*	
			Not straight	O <sup>a</sup>	nN <sup>d</sup>	Puncture each other	
			Not straight	nO <sup>b</sup>	nN <sup>d</sup>	-*	
		Dark Brown	37 - 80	straight	O <sup>a</sup>	N <sup>c</sup>	-*
				Not straight	O <sup>a</sup>	N <sup>c</sup>	-*
				Not straight	O <sup>a</sup>	nN <sup>d</sup>	-*
				Not straight	O <sup>a</sup>	nN <sup>d</sup>	Puncture each other
				Not straight	nO <sup>b</sup>	nN <sup>d</sup>	-*
Reddish Brown	37 - 80	Not straight	O <sup>a</sup>	nN <sup>d</sup>	-*		
		straight	O <sup>a</sup>	N <sup>c</sup>	-*		
		Not straight	O <sup>a</sup>	N <sup>c</sup>	-*		
		Not straight	O <sup>a</sup>	N <sup>c</sup>	-*		
Yellow	37 - 80	straight	O <sup>a</sup>	N <sup>c</sup>	-*		
		Not straight	O <sup>a</sup>	N <sup>c</sup>	-*		
Slightly Greenish Yellow	37 - 80	Not straight	O <sup>a</sup>	N <sup>c</sup>	-*		
		Not straight	O <sup>a</sup>	N <sup>c</sup>	-*		
Single	Transparent	135 - 210	-*	nO <sup>b</sup>	-*	-*	
		37 - 80	-*	nO <sup>b</sup>	-*	-*	

\*Can not be observed clearly; <sup>a</sup>Organized; <sup>b</sup>Not organized; <sup>c</sup>Neat; <sup>d</sup>Not neat.

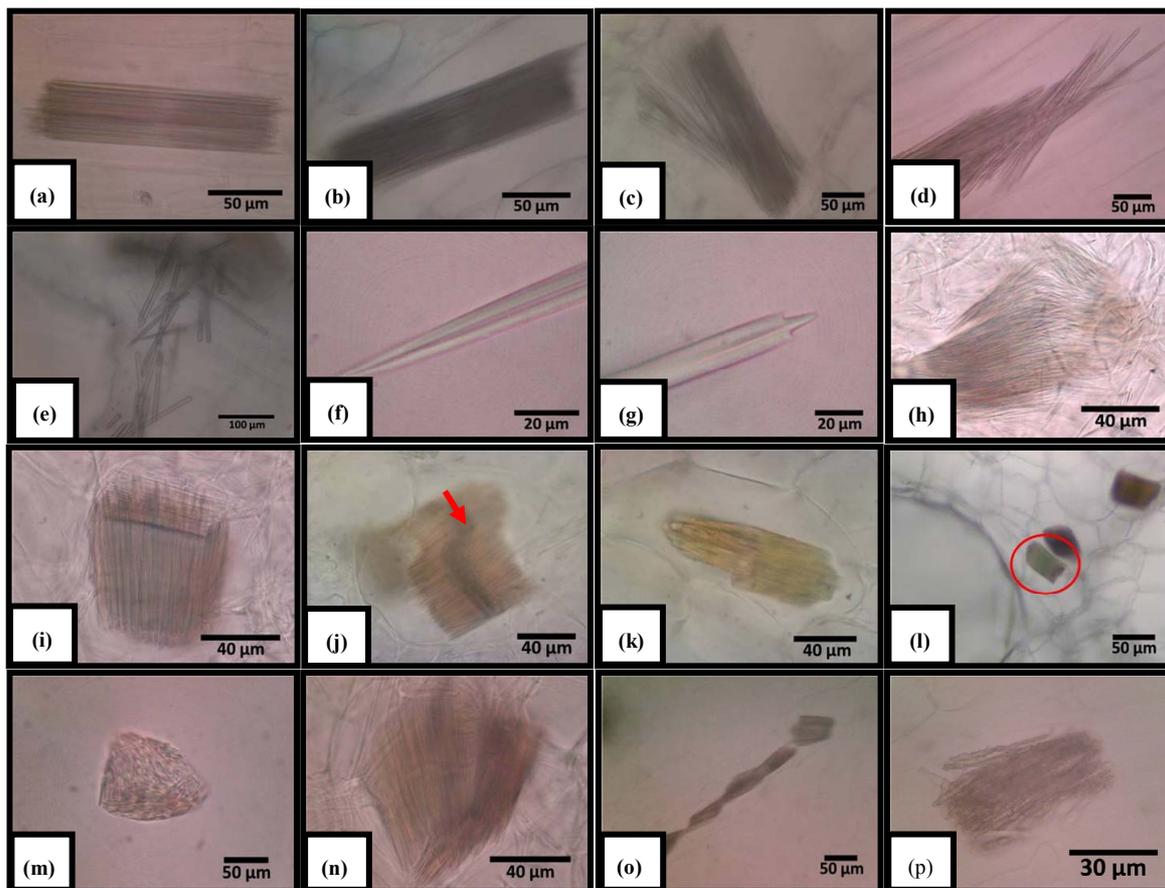
puncture was composed of two or more short raphide crystals that were laid very close so that groups were intersected (stabbed each other) (**Figures 3(h) and (j)**). In addition to bundle form, raphide also had single form. The crystal which had 37 - 80  $\mu\text{m}$  in size was single short raphide crystal which was not form a group and it was spreading. Generally single raphide crystal which had 37 - 80  $\mu\text{m}$  in size based on the observation was not contained in idioblast cell.

By focus on individual crystal, we found three types of crystal end morphology. They include pointed end (**Figure 3(f)**), blunt end, and hook end (**Figure 3(g)**).

Raphide crystals that were found in porang had several different colors. The crystals colors were grouped into dark and bright colors. Crystal colors which were grouped into dark colors *i.e.* dark brown (**Figures 3(h) and (i)**), reddish brown and black colors. Crystal colors which

were grouped into bright colors *i.e.* transparent white, yellow (**Figure 3(k)**), greenish yellow (**Figure 3(l)**), and brown (**Figures 3(j) and (n)**) colors. Long black and short black raphide crystal in the bundle-shaped had black color. Single long and short raphide crystal had a transparent white color. Short brown raphide crystal had several colors *i.e.* greenish yellow, yellow, and light brown colors. Short dark brown raphide crystal also had two colors *i.e.* deep brown and reddish brown colors.

Raphide crystal formation on porang occurs in the early stages of leaf development of organs and tissue differentiation. Generally raphide idioblast was gradually formed and organized, and initiated the formation raphide continuously throughout life of plant tissues. Raphide crystals in *Amorphophallus* could as defend weapon to attacks from herbivorous animals, and contribute to the accumulation of Ca and reabsorption under certain envi-



**Figure 3.** Variation of raphide crystal types in porang: (a) Straight edge long black raphide crystal; (b) Not straight edge long black raphide crystal; (c) Not neatly organized long black raphide crystal; (d) Long black raphide crystal with puncture each other structure; (e) Not organized long black raphide crystal; (f) Single raphide crystal with pattern central line and pointed end; (g) Single raphide crystal with pattern central line and hooked end; (h) Short dark brown raphide crystal with puncture each other structure (formed a fold); (i) Short perfect block shaped dark brown raphide crystals; (j) Short brown raphide crystals with puncture each other structure (formed a belt pattern (arrow)); (k) Not straight edge short yellow raphide crystals; (l) Straight edge short slightly greenish yellow raphide crystal; (m) Spherical shape of short bundle raphide crystal; (n) Short imperfect block shaped brown raphide crystal; (o) Sequenced of five short bundle raphide crystals; (p) Brittle raphide crystal.

ronmental conditions, while the druse crystals only play a role in the buildup process for the exiled (sequestration) [2]. Raphide crystals had a variety of sizes and number, per bundle 100 to 800 single raphide crystal. Single raphide crystals had a constant thickness in the middle and tapered at the edges. Each crystal had two opposing grooves, cavities in almost all parts of the crystal that comes from the center of the crystal. Latch or hook at the end of raphide crystals could be found in some of *Amorphophallus* species [9], one of them was Porang (*Amorphophallus muelleri*).

Raphide crystals that were found in leaf were grouped into bundle and single form. The bundle had two size, namely short (around 75 - 80  $\mu\text{m}$  in size) and long (135 - 250  $\mu\text{m}$  in size). And the single had size 37 - 80  $\mu\text{m}$ . In the leaf, we found unique form *i.e.* sequenced five short crystals (**Figure 3(o)**). The color of raphide crystals in petiole was generally black, dark brown, reddish brown, brown, and transparent.

Raphide crystals that were found in petiole had some similarity form with leaf's crystals. Some crystals seemed broken so that the crystal looks rough, brittle, and frizzy (**Figure 3(p)**). The color of crystal included black, dark brown, brown, and transparent. Such as in leaves, petiole also had varied form and size of crystal. The bundle raphide was categorized short and long. Example short size was in **Figure 3(j)**. The crystal had pretty morphology, *i.e.* flag shape with brown color.

Raphide crystals in tuber were dominated by short-bundle form and both black and brown colors. The bundle had conspicuous morphology, block and spherical shape. The block shape was divided into two types, namely perfectly block-shaped (**Figure 3(i)**) and imperfectly block-shaped (**Figure 3(n)**). The spherical shape

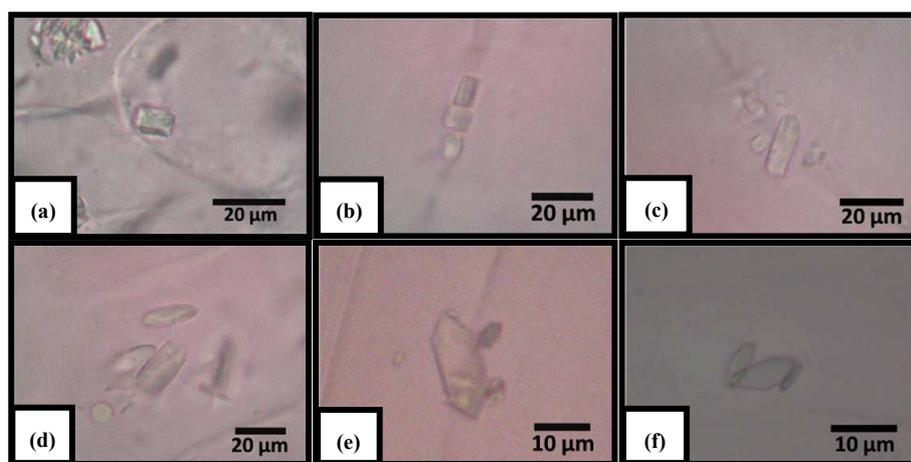
could not divide into certain category because they didn't have various shapes. Example of spherical shape was like "compressed rugby ball" (**Figure 3(m)**). Raphide crystal had some varies color. They were black, dark brown, reddish brown, brown, yellow, slightly greenish yellow and transparent colors. Single raphide crystal in tuber had patterns that were not much different from that were found in leaf and petiole. Although rarely found in tuber, it was recognised single raphide crystals which has a curved shape.

Variations of raphide crystals that were found in this study presumably were caused by genetic influences. Franceschi and Nakata [2] explained that the crystal size variation within a species was influenced by intrinsic factors (genetic factors). In addition to genetic factor, environmental factors also influenced crystal formation. The availability of calcium was example of environmental factor.

### 3.4. Prism Crystal

Prism crystals which were found in porang were transparent white, rectangular shape and composed of six as well as single (**Figure 4(a)**) or in groups (**Figures 4(b)-(d)** and **(f)**) with size 2 - 20  $\mu\text{m}$  (**Table 3**). It was also found that short agglomeration that had 2 - 10  $\mu\text{m}$  in size.

Based on observation, prism crystals that were found in leaf were divided into groups and single prism crystal. it could be composed of single prism crystal with the same shape (**Figure 4(b)**) or different, like a prism crystal group which was composed of single prism crystal 11 - 20  $\mu\text{m}$  in size and single prism 2 - 10  $\mu\text{m}$  in size (**Figure 4(c)**). Single prism crystals that were found in leaf, *i.e.* single prism which had 2 - 10  $\mu\text{m}$  in size (**Figure 4(a)**),



**Figure 4.** Variation of prism crystal types in porang: (a) single prism crystal with size about 2 - 10  $\mu\text{m}$ ; (b) Prism crystal group that were composed of single crystal with size about 2 - 10  $\mu\text{m}$ ; (c) Prism crystal group that were composed of single crystal with size about 2 - 10  $\mu\text{m}$  and single prisms crystal with size about 11 - 20  $\mu\text{m}$ ; (d) Prism crystal group that were composed of single prism crystal with size about 11 - 20  $\mu\text{m}$ ; (e) Hexagon prism crystal; (f) Prism crystal group that were composed of two hexagon crystals.

single prism which had 11 - 20  $\mu\text{m}$  in size, and hexagon prism crystal (**Figures 4(e) and (f)**).

Prism crystals that were found in petiole were divided into single and group of crystal. Single prism crystals that were found in petiole had several different forms, such as single prism crystal which had size 11 - 20  $\mu\text{m}$  and 2 - 10  $\mu\text{m}$ . Prism crystal group that were found in petiole also had several different forms, such as a prism crystal group which was composed of size 2 - 10  $\mu\text{m}$  prism crystals, a prism crystal group which was composed of hexagon prism crystals, and prism crystal groups which was a combination of hexagon prism crystals and size 2 - 10  $\mu\text{m}$  prism crystal.

Prism crystals that were found in tuber had similar variation with the one in petiole and leaf. Therefore it was divided into 3 types of prism crystals, *i.e.* single prism crystal which had 11 - 20  $\mu\text{m}$  in size, slim single prism crystal, and single prism crystal which had 2 - 10  $\mu\text{m}$  in size. Prism crystal group that were found could be composed of single prism crystal with a particular form. The form of prism crystal groups were composed of a slim single prism crystals which had 11 - 20  $\mu\text{m}$  in size and slim single prism crystals which had 2 - 10  $\mu\text{m}$  in size.

Prism crystal that had found in porang had different size with the one in another species from Family Araceae. Some of species from Family Araceae had longer size of prism crystal, *e.g.* prism crystal in *Dieffenbachia seguine* had size of about 48  $\mu\text{m}$  and about 65  $\mu\text{m}$  in *Caladium bicolor* and *Xanthosoma* sp. Prism crystal in *Dieffenbachia seguine*, *Caladium bicolor* and *Xanthosoma* sp could found in inside the thecae, mixed in with the pollen grains [14].

There was also a group called agglomeration prism

crystal (**Figures 5(e) and (f)**) [16]. Named agglomeration prism crystal based on irregular crystalline forms. These crystals were one of the types of crystals that could be found in all plant organs has been observed. Although it could be found throughout of the plant organs, these crystals were present in small amounts. It could be found in a single or a group of crystal. Agglomeration prism crystal had a translucent white color.

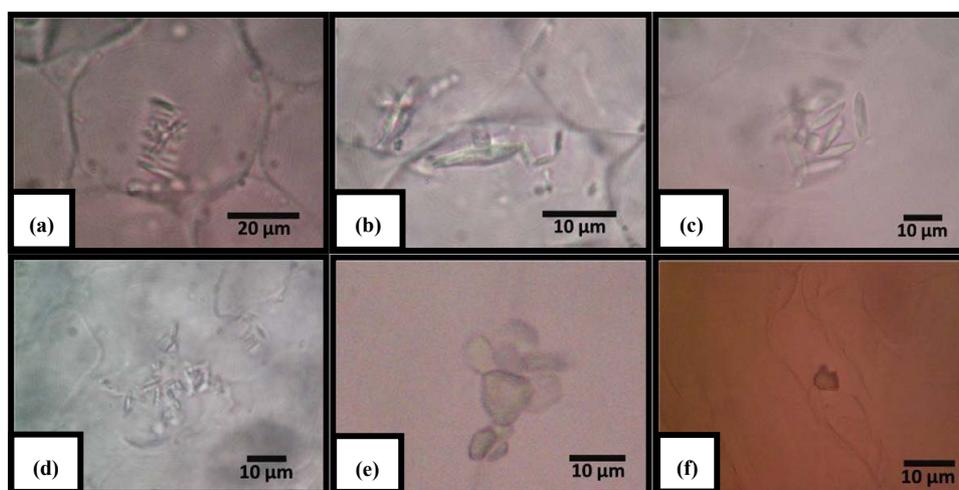
### 3.5. Styloid Crystal

Styloid crystals that contained in porang were transparent white which had a size range of 2 - 13  $\mu\text{m}$  and composed of single or group (**Table 4**). Styloid crystals which were arranged regularly and lined up neatly were generally found in groups. Otherwise, single styloids that were spread were grouped in an irregular arrangement. Styloid crystals that were found in leaf usually present in group. Styloid crystal groups could be found in the form of

**Table 3. Types of crystal prisms in porang (*A. muelleri*).**

Type	Size ( $\mu\text{m}$ )	Angles/ Side
Single	11 - 20	Four
	2 - 10	Four
	11 - 20	Six
Group	11 - 20	Four
	2 - 10	Four
	2 - 10	Six
	2 - 20	-*
Agglomeration	2 - 10	-*

\*Could not be observed clearly



**Figure 5. Other Variation of small crystal types in porang: (a) Regular styloid crystal group with size about 2 - 10  $\mu\text{m}$ ; (b) Styloid crystal group that were composed of styloid with size about 11 - 13  $\mu\text{m}$  and the smaller one (2 - 10  $\mu\text{m}$ ); (c) Irregular styloid crystal group with size about 11 - 13  $\mu\text{m}$ ; (d) Irregular styloid crystal group with size about 2 - 10  $\mu\text{m}$ ; (e) Agglomeration prism crystal group; (f) Single agglomeration prism crystal.**

**Table 4. Types of crystals in porang stiloid (*A. muelleri*).**

Type	Size ( $\mu\text{m}$ )	Position
Single	2 - 10	Irregular
	11 - 13	Irregular
Group	2 - 10	Regular
		Irregular
	11 - 13	Irregular

regular (lined up neatly) (**Figure 5(a)**) and irregular (**Figures 5(b)-(d)**). Styloid crystal group might be composed of crystals that had 2 - 10  $\mu\text{m}$  in size only, styloid crystals 11 - 13  $\mu\text{m}$  in size only, and the combination of both. Styloid crystals were dispersed irregularly in leaf. As the one that had found in leaf, styloid crystals that had found in petiole were also divided into single and group crystal. Generally styloid crystals that were found in tuber always spread and irregular. In tuber, styloid crystals were found in single and group crystal. There was also transparent styloid crystal that was arranged puncture each other in tuber. Grouping styloid crystals could be seen in **Table 4**.

CaOx crystal types that were found in each porang organ were varies. Level of diversity of CaOx crystals that were found was different in each porang organ (**Table 5**).

The highest diversity of druse and raphide crystals was found in tubers, *i.e.* 80% and 91.84%. Different with druse and raphide, the highest prism crystals diversity was found in petiole (100%). However diversity of styloid crystal was equal between leaf and tuber, *i.e.* 80%. From the results of these calculations, it could be concluded that each organ in porang had different types of CaOx crystals morphology.

Differences in level of diversity of each CaOx crystal types in each organ were allegedly due to the environmental factors and genetic regulation that express the diversity of crystal types in each porang organ. In addition, variations of crystal type that were found in porang had some differences with CaOx crystals that were found in other Araceae species. Therefore there were allegations that the variations of crystal type that were found in this research were a hallmark of these porang. As explained by Ilarslan *et al.* [13] and Franceschi and Nakata [2], morphology and distribution of CaOx crystals within a species is influenced by genetic control. Also explained by Franceschi and Nakata [2], determination CaOx crystal types and distribution could be classified as a taxonomic character for species classification.

#### 4. Conclusion

Tuber organ has the highest diversity of crystals compared with other organs, with the percentage of 91.84%

**Table 5. Variability types caox crystals in leaf, petiole, and tuber in porang (*A. muelleri*).**

No	Type of CaOx Crystals	Porang organs		
		Leaf (%)	Petiole (%)	Tuber (%)
1	Rafida ( $n^a$ ) = 49)	59.18	61.22	91.84
2	Druse ( $n^a$ ) = 10)	60	70	80
3	Prisma ( $n^a$ ) = 8)	87.50	100	87.50
4	Stiloid ( $n^a$ ) = 5)	80	60	80

<sup>a</sup>Whole number variation morphology of CaOx crystal, which  $n = 100\%$ .

for rafida, 80% for the Druse, 87.50% for the prism, and 80% for stiloid. In all three organs (leaves, leaf stalks, tubers) of porang plants (*A. muelleri*), four basic shapes of crystals, namely 1) druse, 2) raphide, 3) prism and 4) styloid crystals were obtained. From observations of crystal morphology, raphide crystal variation types of crystal had the highest compared with other crystals. CaOx crystals were divided by size into large crystals (20 - 250  $\mu\text{m}$ ) and small crystal size (1 - 15  $\mu\text{m}$ ).

#### REFERENCES

- [1] D. Barabe, C. Lacroix, M. Chouteau and M. Gibernau, "On the Presence of Extracellular Calcium Oxalate Crystals on the Inflorescences of Araceae," *Botanical Journal of the Linnean Society*, Vol. 146, No. 2, 2004, pp. 181-190. doi:10.1111/j.1095-8339.2004.00318.x
- [2] V. R. Franceschi and P. A. Nakata, "Calcium Oxalate in Plant: Formation and Function," *Annual Review of Plant Biology*, Vol. 56, No. 1, 2005, pp. 41-71. doi:10.1146/annurev.arplant.56.032604.144106
- [3] H. Ilarslan, R. G. Palmer, J. Imsande and H. T. Horner, "Quantitative Determination of Calcium Oxalate and Oxalate in Developing Seeds of Soybean (Leguminosae)," *American Journal of Botany*, Vol. 84, No. 9, 1997, pp. 1042-1046. doi:10.2307/2446147
- [4] C. J. Prychid and P. J. Rudall, "Calcium Oxalate Crystals in Monocotyledons: A Review of Their Structure and Systematics," *Annals of Botany*, Vol. 84, No. 6, 1999, pp. 725-739. doi:10.1006/anbo.1999.0975
- [5] R. C. Keating, "Systematic Occurrence of Raphide Crystals in Araceae," *Annals of The Missouri Botanical Garden*, Vol. 91, 2004, pp. 495-504.
- [6] G. G. Cote, "Diversity and Distribution of Idioblasts Producing Calcium Oxalate Crystals in *Dieffenbachia seguine* (Araceae)," *American Journal of Botany*, Vol. 96, No. 7, 2009, pp. 1245-1254. doi:10.3732/ajb.0800276
- [7] A. Crowther, "Morphometric Analysis of Calcium Oxalate Raphides and Assessment of Their Taxonomic Value for Archaeological Microfossil Studies," Queensland University, Brisbane, 2009, pp. 120-128.
- [8] S. Pitojo, "Suweg," Kanisius, Yogyakarta, 2007.
- [9] C. J. Prychid, R. S. Jabaily and P. J. Rudall, "Cellular

- Ultrastructure and Crystal Development in *Amorphophallus* (Araceae),” *Annals of Botany*, Vol. 101, No. 7, 2008, pp. 983-995. [doi:10.1093/aob/mcn022](https://doi.org/10.1093/aob/mcn022)
- [10] J. M. Genua and C. J. Hillson, “The Occurrence, Type and Location of Calcium Oxalate Crystals in the Leaves of Fourteen Species of Araceae,” *Annals of Botany*, Vol. 56, 1985, pp. 351-361.
- [11] M. H. Grayum, “Evolution and Phylogeny of the Araceae,” *Annals of the Missouri Botanical Garden*, Vol. 77, 1990, pp. 628-697. [doi:10.2307/2399668](https://doi.org/10.2307/2399668)
- [12] S. J. Mayo, J. Bogner and P. C. Boyce, “The Genera of Araceae,” The Trustees, Kew, 1997.
- [13] R. C. Keating, “Leaf Anatomical Characters and Their Value in Understanding Morphoclines in the Araceae,” *The Botanical Review*, Vol. 68, No. 4, 2003, pp. 510-523. [doi:10.1663/0006-8101\(2002\)068\[0510:LACATV\]2.0.CO;2](https://doi.org/10.1663/0006-8101(2002)068[0510:LACATV]2.0.CO;2)
- [14] G. G. Cote, “Distribution of Calcium Oxalate Crystals in Floral Organs of Araceae in Relation to Pollination Strategy,” *American Journal of Botany*, Vol. 99, No. 7, 2012, pp. 1-12. [doi:10.3732/ajb.1100499](https://doi.org/10.3732/ajb.1100499)
- [15] H. Ilarslan, R. G. Palmer and H. T. Horner, “Calcium Oxalate Crystals in Developing Seeds of Soybean,” *Annals of Botany*, Vol. 88, No. 2, 2001, pp. 243-257. [doi:10.1006/anbo.2001.1453](https://doi.org/10.1006/anbo.2001.1453)
- [16] C. Meric, “Calcium Oxalate Crystals in *Aster squamatus* and *Bellis perennis* (Asteraceae: Astereae),” *Phytologia Balcanica*, Vol. 15, No. 2, 2009, pp. 255-259.