Asian Research Journal of Agriculture

10(2): 1-12, 2018; Article no.ARJA.45106
ISSN: 2456-561X

Evaluation of Calcium Regulating Role of Calcium Oxalate Crystals in Eddo Corms in Hydroponic Solution Containing Calcium at Different Concentrations

Mohammad Nazrul Islam[1,2*], Shilpi Islam[2,3] and Michio Kawasaki[2,4]

[1]Phenix Group, House No. 39A, Road No. 8, Dhanmondi, Dhaka, Bangladesh.
[2]The United Graduate School of Agricultural Sciences, Iwate University, 3-18-8 Ueda, Morioka 020-8550, Iwate, Japan.
[3]Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur-1706, Bangladesh.
[4]Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARJA/2018/45106
Editor(s):
(1) Dr. Tancredo Souza, Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Portugal.
(2) Dr. Afroz Alam, Department of Bioscience & Biotechnology, Banasthali University, Rajasthan, India.
Reviewers:
(1) Victor Hugo de Farias Guedes, São Paulo State University, Brazil.
(2) Isela Quintero Zapata, Universidad Autónoma de Nuevo León, Mexico.
Complete Peer review History: http://www.sciencedomain.org/review-history/27680

Original Research Article

Received 15 September 2018
Accepted 27 November 2018
Published 08 December 2018

ABSTRACT

Aims: Involvement of calcium oxalate crystals in the maintenance of calcium homeostasis in the corms of eddo in hydroponic solution containing calcium at different concentrations was investigated.
Study Design: Plants of eddo [*Colocasia esculenta* (L.) Schott var. *antiquorum* Hubbard & Rehder] cv. Aichiwase was used in this study. Seed corms were planted in plastic pots filled with vermiculite and the plants were sprouted by watering under natural temperature, light and humidity conditions in the green house at Hirosaki University, Hirosaki, Japan. After 2 months the plants were grown in a

**Corresponding author: E-mail: nazrulbsmrau@yahoo.com;*

growth chamber. Plants with new growing corms were subjected to grown in water culture solutions containing one of the four treatments as 0 mM calcium, 1 mM calcium nitrate (control), 15 mM calcium nitrate and 15 mM calcium chloride.

Results and Discussion: Under scanning electron microscopy and optical microscopy, few number of raphide crystals were observed in the cortex and many druse crystals were observed in the lateral part containing cortex, the connecting part between root and corm, and the peripheral part of stele. Very few number of druse crystals were observed in the center part of stele. The number and size of crystals in the lateral part of corms under 1 mM calcium nitrate treatment were significantly higher than that in 0 mM calcium solution and were significantly lower than that in the 15 mM calcium solutions. Calcium mapping images by energy dispersive X-ray spectrometry showed a positive correlation between the amounts of calcium in the lateral part of corms and calcium concentration in treatment solutions. The weight percentage of calcium relative to the total weight of major constituent elements per crystal idioblast was significantly higher than that per parenchyma cell in the lateral part. However, the weight percentage of calcium per parenchyma cell except idioblasts of the lateral part was stable among the treatments. The results suggest that calcium is accumulated in crystals under calcium-excessive conditions and is released from crystals under calcium-deficient conditions for stabilisation of calcium levels in the tissues other than the idioblasts in the lateral part of eddo corm. In addition, the weight percentage of calcium per storage parenchyma cell of stele was stable among the treatments and was significantly lower than the weight percentage of calcium per parenchyma cell of the lateral part.

Conclusion: The results indicate that calcium delivered from roots is mostly entered at the lateral parts of corms and is accumulated in calcium oxalate crystals in the cortex, the connecting part between root and corm, and the peripheral part of stele. As a result, calcium contents of the storage parenchyma cells of stele may remain stable at a low level under different calcium treatments.

Keywords: Calcium; calcium oxalate crystal; calcium homeostasis; corm; hydroponic solution; eddo.

1. INTRODUCTION

Taro is the common name for edible aroids which are important staple food in many parts of the world particularly in Asia and the Pacific Islands [1,2]. Taro corm is one of the popular edible root and tuber crops in large parts of Asia, Pacific islands, West Africa and Amazonian regions of South America. Taro corms are high in carbohydrate in the form of easily digested starch and are primarily a source of energy [3,4,5,6] and contain considerable amount (70-80 g/100 g dry taro) of starch [4]. The heart, soft white fleshed corms are highly valued as staple food for human and are eaten boiled, fried, roasted, fermented, or as some taro products such as cake, chips, bread and biscuit prepared from a combination of taro flour and other cereals and grains [7]. The digestibility of taro starch has been estimated to be 98.8%. Therefore, taro flour and other products have been used for infant formulae in the United States and have formed an important constituent of proprietary canned baby foods [7]. Low-grade corms are also used for alcohol production. The mother corms, bad quality corms and peelings can be fed to livestock, mainly cattle and pig [8,9]. Some taro varieties are used only for pigs in Vietnam [10]. *Colocasia esculenta* corm is a potential useful energy supplement in ruminant feeding due to its energy content and proximate metabolisable energy content [11]. Sundried taro meal can replace maize up to 50% in the diet of *Achatina achatina* without adverse effects on reproductive traits [12]. Boiled sun-dried taro cormels can replace maize up to 50% (8.4% of the total diet) in the diets of Japanese quails [13] and boiled peeled sundried taro meal can replace maize in the diets of broiler finishers at 100% inclusion level, without any significant adverse effects on the performance characteristics of the birds [14].

Eddo belongs to the taro group and is mainly cultivated in Asia. Eddo produces a relatively small main corm and many side corms (cormels). However, the corms of taros including eddo contain soluble oxalate and calcium oxalate crystals, which are not good for health and eating quality. The presence of calcium oxalate crystals in the corms has been associated with a painful irritation of mouth and skin which produced on contact and during handling. Calcium oxalate is a major component of kidney stone, and high intake of calcium oxalate may reduce the calcium availability in the body which may be an increased risk factor for women who require a greater amount of calcium in their diets [15]. Oxalates also interfere with the

utilisation of minerals by making them unavailable or reduce in the body [16].

The tubular arrangement of idioblasts containing calcium oxalate crystals localised in the peripheral part of the cortex in the apical zone of the primary roots in eddo [17]. It was also found that there was a positive correlation between calcium concentrations in growth medium and the number and size of the crystals in the apical zone of primary roots in eddo [18]. Additionally, in the apical zone, the percentage of calcium weight per total weight of the major constituent elements in the cortex parenchyma cell did not vary significantly among different calcium concentrations in growth medium. On the other hand, in the root zone apart from the root apex having no crystals, the weight percentage of calcium per cortex parenchyma cell under excess calcium condition in growth medium was significantly higher than that in the lower calcium condition. These results suggested that the crystals in the tubular arrangement participated in the regulation of calcium levels in cortex parenchyma cells in the apical zone of primary roots [18]. Calcium oxalate crystals in the leaf blade and petioles were also participated in the regulation of calcium levels in the mesophyll cells of leaf blade and parenchyma cells of petioles [19]. Very few information are available on the form and distribution of calcium oxalate crystals in taro corm. Earlier only the distribution of calcium oxalate crystal idioblast in the corms of a cultivar of *Colocasia esculenta* known as 'Akalomamale' was investigated [20]. However, there is no information on the calcium regulating role of calcium oxalate crystals in any corms of taros including eddo.

Calcium is an essential element for plant growth and development. It plays important roles, for example, as a structural component of cell wall [21], a signal in various physiological and developmental pathways [22] and an osmoticum [23]. Nonetheless, cytoplasmic concentration of free calcium must be maintained at a suitable level for normal plant growth and development, because excess calcium can be cytotoxic due to its tendency to precipitate with inorganic phosphate [24]. Calcium content of crop plants is also important regarding the nutritional value and quality of human food and/or animal feed [25].

To reveal the mechanism of calcium regulation in the corms under different levels of calcium in growth medium is important for developing strategy of upgrading the growth, the tolerance of environmental calcium stress and the eating quality of eddo corm. The purpose of the study is to elucidate whether and how calcium oxalate crystals play a role in calcium regulation in eddo corms, or not.

2. MATERIALS AND METHODS

2.1 Plant Materials and Treatments

Plants of eddo [*Colocasia esculenta* (L.) Schott var. *antiquorum* Hubbard & Rehder] cv. Aichiwase was used in this study. Seed corms were planted in plastic pots filled with vermiculite and the plants were sprouted by watering under natural temperature, light and humidity conditions in the green house at Hirosaki University (40°59′ N, 140°47′ E, 53 m above sea level), Hirosaki, Japan. After the plants were 15 – 18 cm tall under the condition for 2 months, they were transferred to a water culture solution [17] with continuous aeration. The plants were then grown in a growth chamber (MLR-351H, SANYO) kept at 24ºC, with 18 hours light at about 150 μM m^{-2} s^{-1} (measured by MES-136, KOITO), 6 hours dark and 60% humidity. After the plants were grown for 1 week to about 20 cm tall and with primary roots about 15 cm, they were transferred to tall beakers containing culture solution. Plants with new growing corms were subjected to grow with one of the four treatments as 0 mM calcium, 1 mM calcium nitrate [$Ca(NO_3)_2$] (normal concentration as control), 15 mM calcium nitrate [$Ca(NO_3)_2$] and 15 mM calcium chloride [$CaCl_2$]. Plants were grown in water culture solution in a growth chamber for 7 days [18]. None of the plants used for this treatment showed signs of abnormal growth, stress disorder or a transition to reproductive growth, such as flower-bud formation. After the treatment, the corms were sampled and used for subsequent investigations.

2.2 Calcium Determination

Corms treated with different concentrations of calcium solutions were dried in a drying oven (DV 340S; YAMATO) at 60°C for 3 days to record constant dry weight. The dried corms were ground and stored in a desiccator. Samples were digested with 1% hydrochloric acid for 4 days and diluted with distilled water. To determine the amount of calcium, each sample was analysed by flame atomic absorption spectrophotometer (Z-2000, HITACHI). The

extraction solution contained 1000 ppm lanthanum chloride to prevent disturbances at the time of the spectrophotometry. Corms of six plants per treatment were used for calcium determination.

2.3 Bright-field Optical Microscopy and Measurement of the Number and Size of Crystals

After the treatment, corms were sampled at the lateral part and at the center part of stele and immersed in formalin-acetic acid-alcohol solution (formalin : acetic acid : 70% ethanol = 1 : 1 : 18) at 20°C under reduced pressure for 1 hour and then immersed in the solution at 20°C for 3 days. The corm samples were dehydrated in an ethanol series and then immersed in t-butyl alcohol and embedded in paraffin. Longitudinal sections (20 µm in thickness) were made by using a microtome (RV-240, YAMATO) and then stained and observed under a bright-field optical microscope [18]. The number and size of crystals in 20.86 mm^2 (4.84 mm long × 4.31 mm wide) areas of the longitudinal sections of the lateral part of corm were investigated. The crystal size was calculated by measuring their areas using Image J software. Four corms per treatment and two longitudinal sections of each part per corm were investigated and statistically analysed to compare the number and size of crystals in the lateral part of the corms.

2.4 Scanning Electron Microscopy and Calcium Mapping

The treated corms were sampled at the lateral part containing cortex and peripheral part of stele and at the center part of stele. Then they were freeze-dried under a pressure of 11 Pa at −20ºC for 50 hour by using a vacuum freeze dryer (FDU-1200, EYELA). The freeze dried corm samples were cracked longitudinally. The cracked samples were mounted on stubs with conductive carbon tape, coated with platinum using an auto fine coater (JFC-1600, JEOL) and observed under a scanning electron microscope (JSM-7000F, JEOL) at an accelerating voltage of 5 kV to identify the structure of calcium oxalate crystals. Calcium localisation on the cracked sections of the lateral part and center part of stele of corms were investigated by using a scanning electron microscope attached with an energy dispersive X-ray spectrometer (JED-2300F, JEOL) at an accelerating voltage of 20 kV. Corms from four plants per treatment were observed.

2.5 Measurement Calcium Weight Percentage

The corm samples prepared for the Scanning Electron Microscopic (SEM) observation and the calcium mapping were also used for this measurement. The percentage of calcium weight per total weight of the major constituent elements [18] per parenchyma cell and per crystal idioblast on the sections of the lateral part of corms was measured by using a SEM attached with an energy dispersive X-ray spectrometer at an accelerating voltage of 20 kV. The percentage per storage parenchyma cell of stele on the sections of the center part of stele was also measured. The analysis areas for this measurement were about 65 μm^2 (10.4 µm in length and 6.25 µm in width) in the parenchyma cells and crystal idioblasts of the lateral parts and the storage parenchyma cells in the center part of stele of corms. In the idioblasts, not only the crystal but also the cytosol was contained within the analysis area. Two parenchyma cell near the crystal idioblast and two crystal idioblasts per section of the lateral part of corms were measured. Two storage parenchyma cell of stele on the sections of the center part of stele of corms were also measured. Corms of four plants per treatment were investigated.

2.6 Statistical Analysis

Analysis of variance followed by Tukey's test was performed on the data of dry weight, calcium content, number and size of crystals, and weight percentage of calcium per cell.

3. RESULTS

3.1 Dry Weight of Corms

Dry weight (g $plant^{-1}$) of corms under the 15 mM calcium treatments were slightly higher, but no significant difference was observed in dry weight of corms among the treatments (Fig. 1).

3.2 Calcium Contents of Corms

Calcium contents (mg g^{-1} dry weight and mg $plant^{-1}$) in corms from 1 mM calcium nitrate treatment were significantly ($P < 0.01$) higher than that in 0 mM calcium treatment and was significantly ($P < 0.01$) lower than that in 15 mM calcium nitrate and 15 mM calcium chloride treatments (Fig. 2). No significant difference was observed in calcium contents of corms between the two 15 mM calcium treatments.

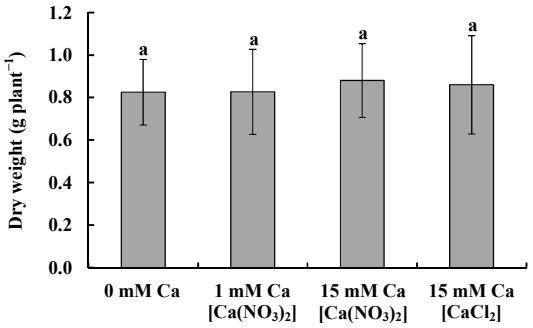

Fig. 1. Dry weight of corms

Same letter indicates no significant difference among the treatments (n = 6, Tukey's test)

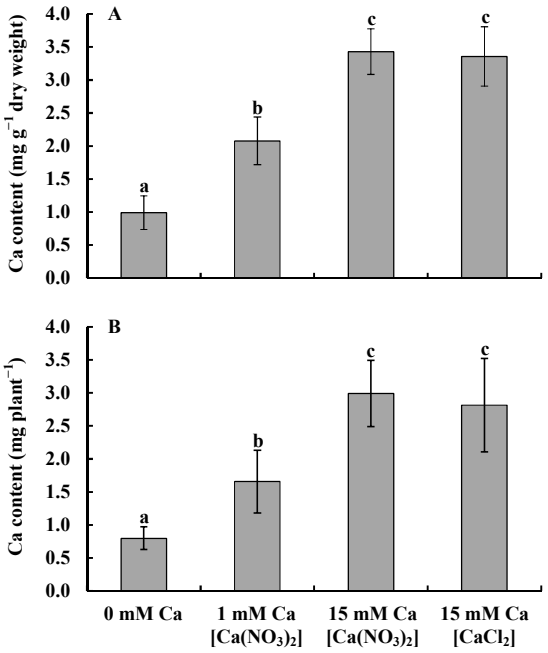

Fig. 2. Calcium contents of corms

Calcium content of corms (A) and total calcium content of whole corm per plant (B). Different letters indicate significant differences at 1% level (n = 6, Tukey's test)

3.3 Distribution and Structure of Calcium Oxalate Crystals

Optical microscopy was used to investigate the differences in crystal distribution and morphology among the treatments. Two forms of calcium oxalate crystals were observed in the corm of eddo under optical microscopy: raphides and druses (Fig. 3A). Raphide crystals were observed in the cortex of corms and druse crystals were observed in the cortex and stele of corms (Fig. 3A). Most of the crystals were druse type and very few were raphide type crystals in the corms of all treatments. Druse crystals were found at high concentrations in the connecting part between root and corm in the peripheral part of stele (Fig. 6). The number and size of crystals in the sections of the lateral part containing cortex and peripheral part of stele of corms in 1 mM calcium treatment were smaller than in 15 mM calcium nitrate and 15 mM calcium chloride treatments and were larger than in 0 mM calcium treatment (Fig. 4). Under optical microscopy, few druse type calcium oxalate crystals were observed in the center part of stele of corms (Fig. 5).

Scanning electron microscopy was used to investigate the fine structure of the crystals in the corms. In the lateral part of corms, raphide crystals were present as the bundle of needles (Fig. 3B) and druse crystals were tetrahedral form (Fig. 3C). Crystals in the lateral part of corms were seemed to be increased in size with increasing the calcium concentration in treatment solutions.

3.4 Number and Size of Crystals

The number and size of calcium oxalate crystals were investigated with using optical microscopic images of corms. The number and size of crystals in the lateral part (containing cortex and peripheral part of stele) of corms in 1 mM calcium nitrate treatment were significantly (P <0.01) greater than that in 0 mM calcium treatment and were significantly (P < 0.01) smaller than that in 15 mM calcium nitrate and 15 mM calcium chloride treatments (Fig. 7). No significant difference was found in crystal number and size in corms between the 15 mM calcium treatments.

3.5 Calcium Localisation

The SEM images and energy dispersive X-ray spectrometric calcium mapping images of the cracked longitudinal sections of the lateral part containing cortex and peripheral part of stele and center part of stele of corms are shown in Fig. 8. Red dots indicate the presence of calcium. In the sections of the lateral part of corms, red dots was intense in the crystal idioblasts. The intensity of dots was highest in the sections under 15 mM calcium nitrate and 15 mM calcium chloride treatments, followed by that of the 1 mM calcium nitrate and 0 mM calcium treatments. In 0 mM calcium treatment, the intensity of dots in crystal idioblasts was the

lowest among the treatments. The pattern of calcium distribution in the parenchyma cells of the lateral part of corms was nearly uniform across the treatments. In the center part of stele of corms, calcium was uniformly distributed in the storage parenchyma cells and the distribution pattern was almost similar in all treatments.

3.6 Weight Percentage of Calcium per Cell in Corms

In the lateral part of corms, the weight percentage of calcium per parenchyma cell and per crystal idioblast did not differ significantly among the treatments (Fig. 9A). However, the weight percentage of calcium per crystal idioblast was significantly ($P < 0.001$) higher than that per parenchyma cell of the lateral part of corms across the treatments (Fig. 9A). The weight percentage of calcium per storage parenchyma cell of center part of stele did not vary among the treatments (Fig. 9B). But, the weight percentage of calcium per storage parenchyma cell of center part of stele was significantly ($P < 0.001$) lower than that per parenchyma cell of the lateral part of corms in all treatments (Fig. 9B).

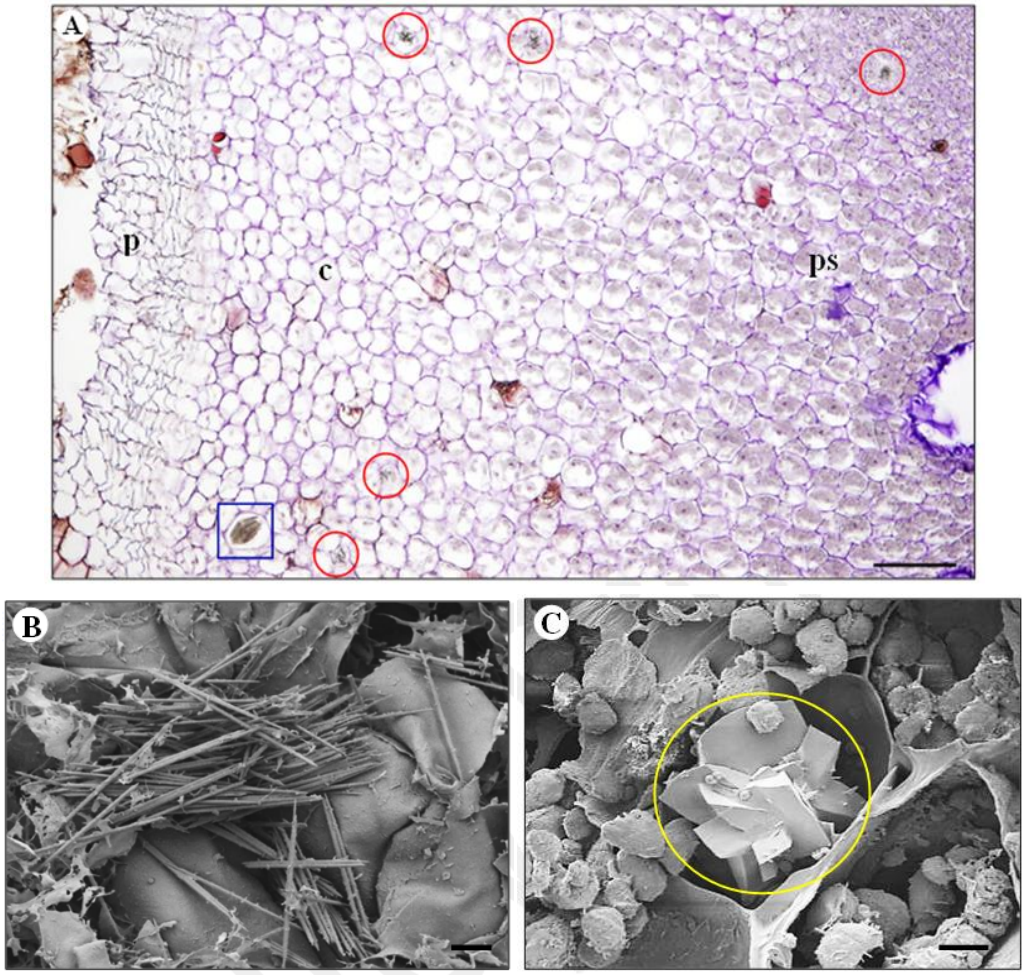

Fig. 3. Calcium oxalate crystals in longitudinal sections of the lateral part of corm

(A) Longitudinal section of corm observed under an optical microscope. Raphide crystal is enclosed in square and the druse crystals are surrounded by circles. c, cortex; p, periderm; ps, peripheral part of stele. Bar = 200 µm.

(B) Raphide crystal in the cracked longitudinal section of the lateral part of corm observed under a scanning electron microscope. Bar = 10 µm.

(C) Druse crystal in the cracked longitudinal section of the lateral part of corm observed under a scanning electron microscope. Bar = 10 µm.

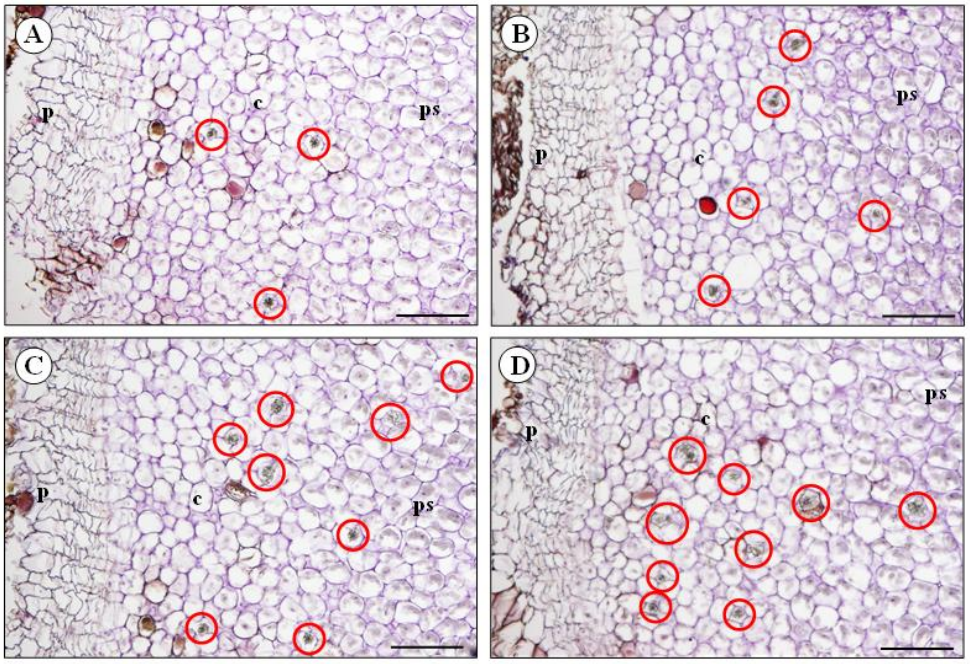

Fig. 4. Longitudinal sections of the lateral part of corms observed under an optical microscope

(A) 0 mM Ca treatment, (B) 1 mM Ca [$Ca(NO_3)_2$] treatment, (C) 15 mM Ca [$Ca(NO_3)_2$] treatment and (D) 15 mM Ca [$CaCl_2$] treatment. Crystals were surrounded with circles. c, cortex; p, periderm; ps, peripheral part of stele. Bars = 200 µm

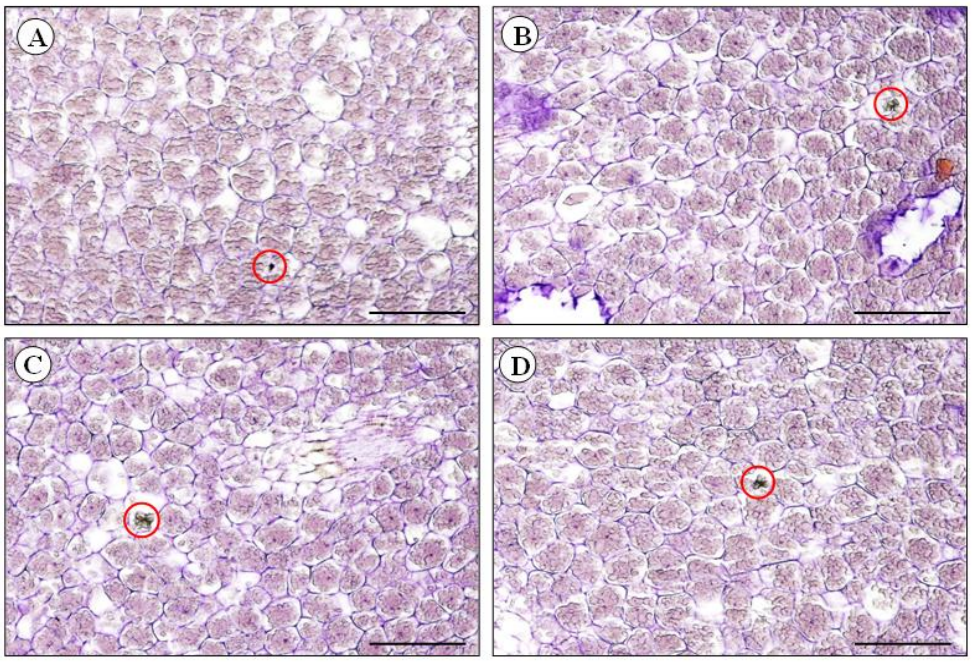

Fig. 5. Longitudinal sections of the center part of stele of corms observed under an optical microscope

(A) 0 mM Ca treatment, (B) 1 mM Ca [$Ca(NO_3)_2$] treatment, (C) 15 mM Ca [$Ca(NO_3)_2$] treatment and (D) 15 mM Ca [$CaCl_2$] treatment. Crystals were surrounded with circles. Bars = 200 µm

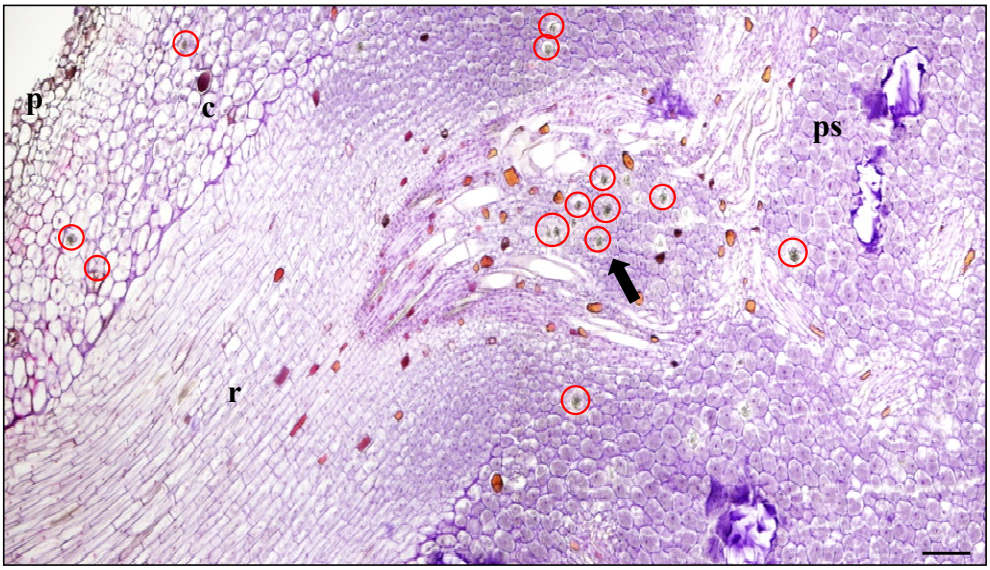

Fig. 6. Distribution of druse crystals in longitudinal section of the lateral part of corm observed under an optical microscope

Druse crystals are surrounded by circles. c, cortex; p, periderm; r, root; ps, peripheral part of stele. ➡ indicates the border between root trace part and corm. Bar = 200 µm

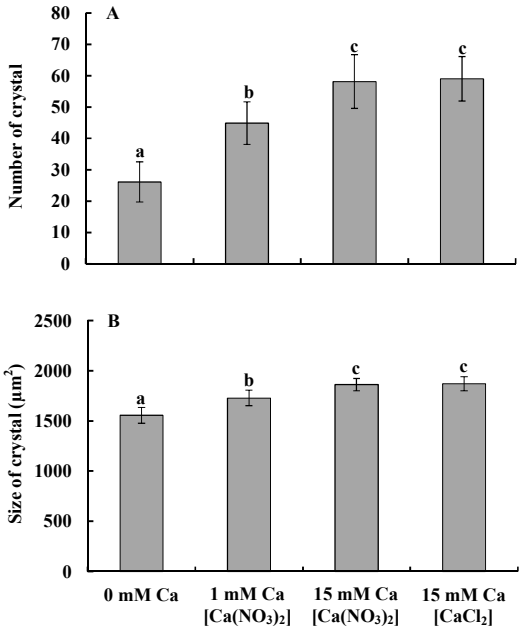

Fig. 7. Number and size of calcium oxalate crystal in the lateral part of corms

The number (A) and size (B) of crystals were investigated in an area of 20.86 mm² (4.84 mm in length and 4.31 mm in width) in longitudinal sections of the lateral part of corms. Four corms per treatment and two sections per corm were investigated. Different letters indicate significant differences at 1% level (n = 8, Tukey's test); error bar defines standard deviation

4. DISCUSSION

Calcium contents (mg g^{-1} dry weight and mg $plant^{-1}$) of corms were increased with increasing calcium concentration in the culture solutions (Fig. 2). These results indicate that the experimental treatments were able to alter the calcium contents of corms. Dry weight of corms did not differ under different calcium concentration treatments (Fig. 1). This indicates that plant size is almost uniform in different treatments and it does not effect in the other results of this study.

Some previous studies showed a positive correlation between the calcium concentration in growth medium and crystal formation in the aerial parts of the plants such as leaves of *Pistia stratiotes* [26], *Phaseolus vulgaris* [27], *Lemna minor* [28], *Morus australis* Poir [29], *Corchorus olitorius* and *Malva parviflora* [30]. In a study with eddo roots subjected to different calcium concentrations, the number and size of crystals in the root apical zone under 1 mM calcium nitrate solution were significantly larger than those in 0 mM calcium solution and were significantly smaller than those in solutions containing 15 mM calcium [18]. Similar results were also observed in another study involving the leaf blades and petioles of eddo under different calcium concentrations in treatments [19]. In this study, calcium oxalate crystals

mostly presented in the lateral parts (cortex and peripheral part of stele) and very few crystals were observed in the center part of stele. Therefore, crystal variability among the treatments was investigated only in the lateral part of corms. Scanning electron microscopy and optical microscopy showed that the number and size of crystals were larger in the lateral part of corms under 1 mM calcium nitrate treatment than that in 0 mM calcium treatment but smaller than that under the 15 mM calcium treatments. In addition, it was confirmed by statistical analysis that both the number and size of crystals in the corms of 1 mM calcium nitrate treatment were significantly higher than that in 0 mM calcium treatment and lower than that of the 15 mM calcium treatments (Fig. 7). These results demonstrate that the crystal variability in the lateral part of corms is directly responsive to the calcium concentration of growth medium.

In this study, calcium mapping images revealed that the calcium was most abundant in crystal idioblasts in the lateral part of corms subjected to the 15 mM calcium treatments (Fig. 8). However, the pattern of calcium distribution in parenchyma cells of the lateral parts of corms was nearly uniform across the treatments (Fig. 8). In an earlier study involving the apical zone of primary roots of eddo having calcium oxalate crystals [18], the weight percentage of calcium relative to the total weight of major constituent elements per crystal idioblast was noticeably higher than the weight percentage of calcium per cortical parenchyma cell, but the weight percentage of calcium per cortical parenchyma cell did not vary significantly among different calcium concentrations in the growth medium. Similar results were observed in another study involving the leaf blades and petioles of eddo under different calcium concentrations in treatments [19]. The weight percentage of calcium per crystal idioblast was noticeably higher than the weight percentage of calcium per mesophyll cell of leaf blades and per normal parenchyma cell of petioles. Although the total calcium contents of leaf blades and petioles

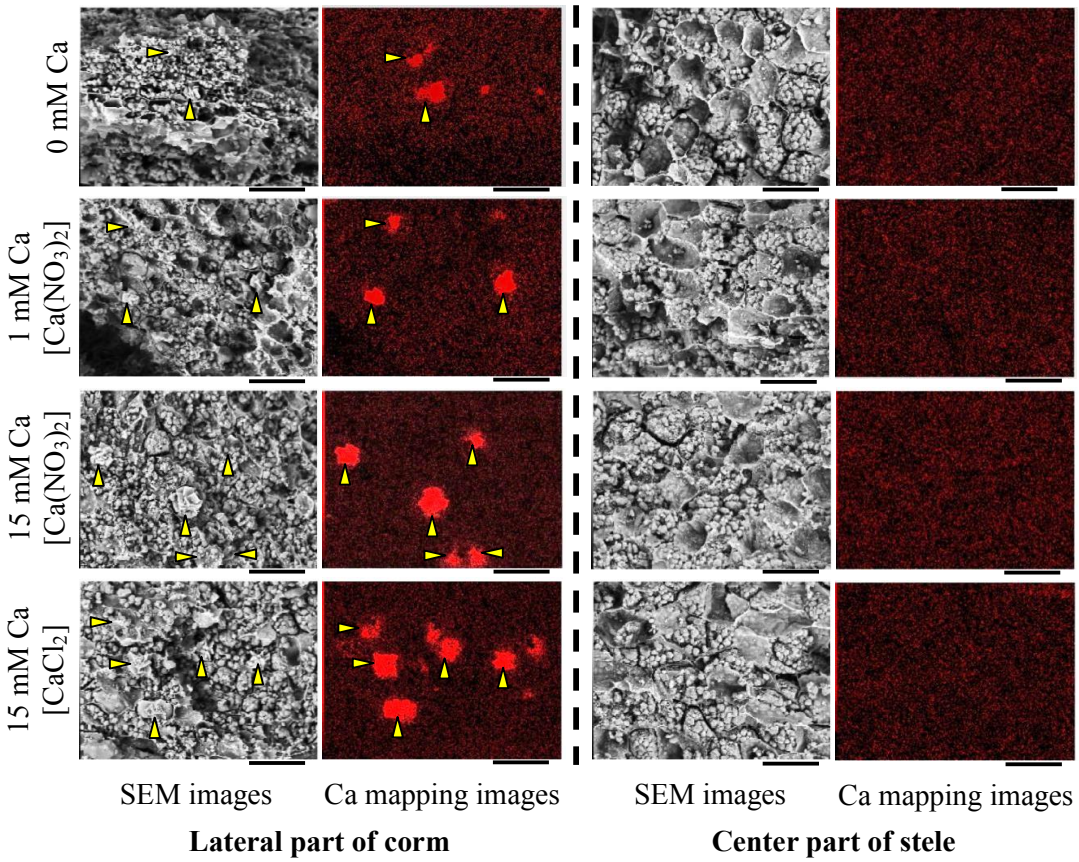

Fig. 8. SEM and calcium mapping images of the longitudinal cracked sections of corms

Red color dots indicate the presence of calcium. Arrowheads indicate crystal idioblasts. Bars = 200 µm

were increased with increasing the calcium concentration in treatments, the weight percentage of calcium per mesophyll cell of leaf blades and per normal parenchyma cell of petioles was stable among the treatments with different calcium concentrations in the growth medium [19]. In the present study, the weight percentage of calcium per crystal idioblast was noticeably higher than the weight percentage of calcium per parenchyma cell of the lateral parts of corms (Fig. 9A). On the other hand, the weight percentage of calcium per parenchyma cell of the lateral parts of corms was stable among the treatments (Fig. 9A). On the basis of these results, it is suggested that calcium accumulates in crystals under calcium-excessive conditions and is released from crystals under calcium-deficient conditions for stabilisation of calcium levels in the tissues other than the idioblasts in the lateral part of eddo corm.

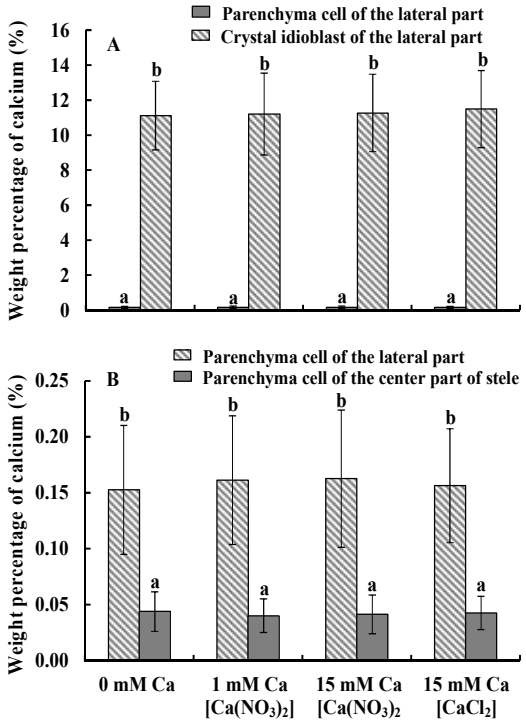

Fig. 9. Weight percentage of calcium per cell in corms

The weight percentage of calcium per parenchyma cell and per crystal idioblast in the lateral part of corms (A) and the weight percentage of calcium per parenchyma cell of the lateral part and per parenchyma cell of the center part of stele of corms (B) were investigated by EDS. Different letters indicate significant differences at 0.01% level (n = 8, Tukey's test); error bar defines standard deviation

In this study, very few crystals were observed in the center part of stele of corms under optical microscopy. Calcium was uniformly distributed in the storage parenchyma cells of stele and the distribution pattern was almost similar across the treatments (Fig. 8). In the stele of corms, the weight percentage of calcium per storage parenchyma cell was also stable among the treatments (Fig. 9B). However, the weight percentage of calcium per storage parenchyma cell of the center part of stele was significantly lower than that of the weight percentage of calcium per parenchyma cell of the lateral parts of corms (Fig. 9B). The results indicate that stele of corm has lower calcium content and the contents do not change in the high calcium concentration treatments. However, the total calcium content of corm was significantly increased with increasing the calcium concentrations in the treatment solutions (Fig. 2B). It is suggested that excess calcium delivered from roots is mostly accumulated in the lateral parts of corms and the size and number of crystal idioblasts were increased. Therefore, few amount of calcium present in the stele of corms.

5. CONCLUSION

From the results of this study it is concluded that calcium delivered from roots is mostly accumulated at the lateral parts of corms and calcium oxalate crystals in the cortex, the connecting part between root and corm, and the peripheral part of stele would play a role in the regulation of calcium levels in the parenchyma cells of the lateral parts. As a result, calcium contents of the storage parenchyma cells of stele would remain stable at a low level under different calcium treatments.

This is the first investigation on the calcium regulating role of calcium oxalate crystals in the corms of eddo grown hydroponically under different calcium concentrations. The knowledge generated by this study should contribute to elucidate the overall mechanism of calcium regulation in eddo for leading to improvements in growth and eating quality of corms.

ACKNOWLEDGEMENTS

We would like to thank Yusei Tsushima, Center for instrumental analysis, Hirosaki University, for providing technical assistance for the energy dispersive X-ray spectrometry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Catherwood DJ, Savage GP, Mason SM, Scheffer JJC, Douglas JA. Oxalate content of cormels of Japanese taro (*Colocasia esculenta* (L.) Schott) and the effect of cooking. Journal of Food Composition and Analysis. 2007;20:147-151.
2. Savage GP, Mårtenson L, Sedcole JR. Composition of oxalate in baked taro (C*olocasia esculenta* var. Schott) leaves cooked alone or with addition of cows milk or coconut milk. Journal of Food Composition and Analysis. 2009;22(1):83-86.
3. Niba LL. Processing effects on susceptibility of starch to digestion in some dietary starch sources. International Journal of Food Science and Nutrition. 2003;54(1):97-109.
4. Tattiyakul J, Asavasaksaku S, Pradipasena P. Chemical and physical properties of flour extracted from taro *Colocasia esculenta* (L) Schott grown in different regions of Thailand. ScienceAsia. 2006;32:279-284.
5. Ndabikunze BK, Talwana HAL, Mongi RJ, Issa-Zacharia A, Serem AK, Palapala V, Nandi JOM. Proximate and mineral composition of cocoyam (*Colocasia esculenta* L. and *Xanthosoma sagittifolium* L.) grown along the Lack Victoria basin in Tanzania and Uganda. African Journal of Food Science. 2011; 5(4):248-254.
6. Adejumo IO, Babalola TO, Alabi OO. *Colocasia esculenta* (L.) Schott as an alternative energy source in animal nutrition. British Journal of Applied Science and Technology. 2013;3(4): 1276-1285.
7. Darkwa S, Darkwa AA. TARO "*Colocasia esculenta*": It's utilization in food products in Ghana. Journal of Food Processing and Technology. 2013;4(5):225. DOI: 10.4172/2157-7110.1000225
8. Carpenter JR, Steinke WE. Animal Feed. Taro. A Review of *Colocasia esculenta* and its Potentials. In J. Wang Ed., University of Hawaii Press, Honolulu. 1983;269-300.
9. Heuzé V, Tran G, Hassoun P, Renaudeau D. Taro (*Colocasia esculenta*). Feedipedia.org. A programme by INRA, CIRAD, AFZ and FAO; 2013. Available:http://www.feedipedia.org/node/537
10. Toan NH, Preston TR. Taro as a local feed resource for pigs in small scale household condition. Livestock Research and Rural Development. 2010;22. Article 152.
11. Babayemi OJ, Bankole MA. Nutrient value and in vitro gas production of African wild cocoyam (*Colocasia esculentum).* African Journal of Food, Agriculture, Nutrition and Development. 2009;9(1):593-607.
12. Okon B, Ibon LA, Nsa EE, Ubua JA. Reproductive and growth traits of parents and F1 hatchings of *Achatina achatina* (L.) snails under mixed feeding regime with graded levels of swamp taro cocoyam (*Cyrtosperma chamissonis*) and pawpaw leaves (*Carica papaya).* Journal of Agricultural Science. 2012;4(11):289-298.
13. Okon BI, Obi MB, Ayuk AA. Performance of quails (*Coturnix coturnix japonica*) fed graded levels of boiled sun-dried taro cocoyam (*Colocasia esculenta*) as a replacement for maize. Agricultural Journal. 2007;2(6):654-657.
14. Ologhobo AD, Adejumo IO. Effects of differently processed [*Colocasia esculenta* (L.) Schott] on growth performance and carcass characteristics of broiler finishers. International Journal of AgriScience. 2011; 1(4):244-248.
15. Singh AK, Chaurasiya AK, Mitra S. Determination of antinutritional changes in elephant foot yam (*Amorphophallus paeoniifolius* Dennst-Nicolson) cultivars. Chemical Science Review and Letters. 2018;7(25):19-24.
16. Singh AK, Chaurasiya AK, Mitra S. Assesment of antinutritional changes in elephant foot yam (*Amorphophallus paeoniifolius* Dennst-Nicolson) cultivars. Vegetable Science. 2017;44(2):57-61.
17. Kawasaki M, Takatsuji A, Taniguchi M, Miyake H. Localization of casparian bands and crystal cells in relation to aluminum distribution in the primary root of eddo under aluminum treatment. Plant Production Science. 2008;11(2): 238-242.
18. Islam MN, Kawasaki M. Morphological changes and function of calcium oxalate crystals in eddo roots in hydroponic

solution containing calcium at various concentrations. Plant Production Science. 2014;17(1):13-19.
19. Islam MN, Kawasaki M. Evaluation of calcium regulating roles of guttation and calcium oxalate crystals in leaf blades and petioles of hydroponically grown eddo. Plant Production Science. 2015;18(1):11-21.
20. Sunell LA, Healey PL. Distribution of Calcium oxalate crystal idioblasts in corms of taro (*Colocasia esculenta*). American Journal of Botany. 1979;66(9): 1029-1032.
21. Demarty M, Morvon C, Thellier M. Calcium and the cell wall. Plant Cell and Environment. 1984;7(6):441-448.
22. Bush DS, Calcium regulation in plant cells and its role in signaling. Annual Review of Plant Physiology and Plant Molecular Biology. 1995;46:95-122.
23. Kinzel H. Calcium in the vacuoles and cell walls of plant tissue. Flora. 1989;182(1): 99-125.
24. Marschner P. Mineral Nutrition of Higher Plants, 3rd edition. Academic Press, San Diego. 2012;409-472.
25. Bhat MS, Shaheen M, Zaman R, Muhee A. Mineral inter-relationship among soil, forage and dairy cattle in Kashmir, India. Veterinary World. 2011;4(12):550-553.
26. Volk GM, Lynch-Holm VJ, Kostman TA, Goss LJ, Franceschi VR. The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in *Pistia stratiotes* leaves. Plant Biology. 2002;4(1):34-45.
27. Kuo-Huang LL, Zindler-Frank E. Structure of crystal cells and influences of leaf development on crystal development and vice versa in *Phaseolus vulgaris* (Leguminosae). Botanical Acta. 1998;111: 337-345.
28. Mazen AMA, Zhang D, Franceschi VR. Calcium oxalate formation in *Lemna minor*: physiological and ultrastructural aspects of high capacity calcium sequestration. New Phytologist. 2004;161(2):435-448.
29. Wu CC, Chen SJ, Yen TB, Kuo-Huang LL. Influence of calcium availability on deposition of calcium carbonate and calcium oxalate crystals in the idioblasts of *Morus australis* Poir. leaves. Botanical Studies. 2006;47:119-127.
30. Faheed F, Mazen A, Elmohsen SA. Physiological and ultrastructural studies on calcium oxalate crystal formation in some plants. Turkish Journal of Botany. 2013;37:139-152.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27680