Evaluation of Calcium Regulating Roles of Guttation and Calcium Oxalate Crystals in Leaf Blades and Petioles of Hydroponically Grown Eddo

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Abstract: We investigated the involvement of guttation and calcium oxalate crystals in the maintenance of calcium homeostasis in leaf blades and petioles of eddo in hydroponic solution containing calcium at different concentrations. Under scanning electron microscopy, two types of crystals were observed in leaf blades and petioles: raphides (bundles of needle-shaped crystals) and druses (multifaceted conglomerate crystals). The number and size of crystals of leaf blades and petioles in 1 mM calcium treatment (control) were smaller than that in 15 mM calcium treatments and larger than that in 0 mM calcium treatment. Calcium contents of leaf blades, petioles and whole plants increased with the increase of calcium concentration in the treatment solution. In addition, calcium-mapping images demonstrated a positive correlation between the amount of calcium in crystal idioblasts and concentration of calcium in treatment solutions. On the other hand, the weight percentage of calcium per mesophyll cell (spongy cell and palisade cell) of leaf blades and per normal parenchyma cell of petioles was stable irrespective of calcium treatment conditions. These results suggest that calcium accumulates in crystals under calcium-excessive conditions and is released from crystals under calcium-deficient conditions to stabilize calcium levels in leaf tissues other than the idioblasts. A positive correlation was observed between the calcium concentration of guttation fluid (μg mL⁻¹), the total amount of calcium in guttation fluid (μg leaf⁻¹ night⁻¹) and the calcium concentration of treatment solutions. These results suggest that guttation eliminates excess calcium and would be involved in maintaining calcium ion homeostasis in eddo.

Key words: Calcium, Calcium oxalate crystal, Eddo, Guttation, Leaf blade, Petiole.

Eddo [Colocasia esculenta (L.) Schott var. antiquorum Hubbard & Rehder] belongs to taro and is mainly cultivated in Asia. The corms of taro are highly valued as a staple food. Additionally, in some countries such as Laos, Cambodia (Buntha et al., 2008) and Vietnam (Hang et al., 2011), taro leaf blades and petioles are used as animal feed. In central Vietnam, some taro cultivars are used only for pigs; other cultivars are harvested to produce corms for human consumption, the petioles and leaves being used as by-product feed for pigs (Toan and Preston, 2010). Taro petioles and leaf blades could potentially be used as forage for livestock in countries where these plants are cultivated.

Most taro cultivars contain numerous calcium oxalate crystals (Bradbury and Nixon, 1998). Calcium oxalate crystals are widely distributed in plants, being present in over 215 higher plant families (Arnott and Pautard, 1970; Kuo-Huang et al., 2002; Nakata, 2003; Franceschi and Nakata, 2005; Kuo-Huang et al., 2007; Mukherjee and Nordenstam, 2010). On the basis of their morphology, plant crystals can be classified into five categories: crystal sand, raphide, druse, styloid and prismatic types (Franceschi and Horner, 1980; Katayama et al., 2007; He et al., 2012). These crystals are formed within the vacuoles of specialized cells called crystal idioblasts (Franceschi and Nakata, 2005). Various functions have been proposed for calcium oxalate crystals in plants including regulation of calcium levels in plant, protection against herbivory, detoxification of heavy metals, tissue strengthening and the gathering and reflection of light for photosynthesis (Prychid and Rudall, 1999; Franceschi, 2001; Molano-Flores, 2001; Nakata, 2003; Franceschi and Nakata, 2005; Nakata, 2012; Faheed et al., 2013).
Previous studies with some plant species have shown that the number and/or size of calcium oxalate crystals are positively correlated with the amount of calcium applied to the growth medium (Kuo-Huang and Zindler-Frank, 1998; Volk et al., 2002; Mazen et al., 2004; Faheed et al., 2013). Wu et al. (2006) also reported that the average distribution density of calcium oxalate crystals was higher in the leaves of *Morus australis* Poir grown in a solution containing a high concentration of calcium and no calcium oxalate crystals were found in the leaves of plants grown in a low calcium solution. These results suggested that calcium accumulates in crystals under calcium-excessive conditions and is released from crystals under calcium-deficient conditions for stabilization of biologically active calcium levels in plants. Kawasaki et al. (2008) reported the tubular arrangement of idioblasts containing calcium oxalate crystals localized in the cortex periphery in the apical zone of primary roots of eddo. Islam and Kawasaki (2014) found a positive correlation between calcium concentration of the growth medium and the abundance and size of crystals in eddo root apical zones. They also found that, the weight percentage of calcium relative to the total weight of major constituent elements in apical zone parenchyma cells did not vary significantly with the calcium concentration in the growth medium. However, in the root zone apart from the root apex which has no crystals, the weight percentage of calcium per cortical parenchyma cell was significantly higher in growth medium with excess calcium than in that with lower calcium concentrations. These results suggest that the tubularly arranged crystals participate in the regulation of calcium levels in cortical parenchyma cells in the apical zone of primary roots (Islam and Kawasaki, 2014). Nevertheless, no experimental evidence exists concerning calcium regulation of crystals in leaf blades and petioles of eddo, even though knowledge regarding this process is needed for understanding the inclusive mechanism of calcium regulation in eddo.

Guttation is a conspicuous visible phenomenon occurring in a wide range of plants (Singh and Singh, 2013). In this process, liquid is exuded from hydathodes situated along margins and on the tips and adaxial and abaxial surfaces of leaves (Singh, 2014). In some plants, guttation fluids contain various salts, amino acids, proteins and sugars (Curtis, 1943; Ivanoff, 1963; Goateley and Lewis, 1966; Curtis and Lersten, 1974; Dieffenbach et al., 1980; Komarnytsky et al., 2000; Mizuno et al., 2002; Pilot et al., 2004; Chen and Chen, 2007; Shapira et al., 2013). Although experimental support is limited, guttation may play a role in the maintenance of plant ionic homeostasis, as salts may be eliminated from hydathodes via their dissolution in guttation fluid (Tester and Davenport, 2003). In taros, a large volume of guttation fluid exudes through the leaf blade hydathodes (Moore et al., 2003). Nevertheless, the relationship between guttation and the maintenance of calcium ion homeostasis in eddo and other taros has not been reported.

Calcium is an essential plant macronutrient, influences many biochemical and physiological processes in plant tissues and cells (Bush, 1995). Although calcium deficiency is rare in nature, excess calcium can be cytotoxic because of its tendency to precipitate with inorganic phosphate (White and Broadley, 2003; George et al., 2012). For normal plant growth, the maintenance of suitable levels of calcium ion in cytoplasm is essential to avoid the effects of calcium deficiency and toxicity (George et al., 2012). The calcium content of crop plants is also an important determinant of the nutritional value and quality of human food and/or animal feed (Bhat et al., 2011).

Elucidation of the mechanism related to calcium regulation in leaf blades and petioles of eddo is needed to develop strategies for enhancing the growth, the tolerance to environmental calcium stress and the eating quality especially for livestock. The purpose of this study was to reveal whether calcium oxalate crystals and guttation play a role in calcium regulation in leaf blades and petioles of eddo, or not. To accomplish this goal, we used optical microscopy and scanning electron microscopy to investigate the distribution, morphology and quantity of crystals in the leaf blades and petioles of eddo under different concentrations of calcium in hydroponic solutions. In addition, calcium localization and weight percentage of calcium relative to the total weight of major constituent elements in cell were investigated by using energy dispersive X-ray spectrometry. We also measured the calcium content in leaf blades, petioles, whole plants and guttation fluid by flame atomic absorption spectrophotometry.

**Materials and Methods**

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 in June 2012, and the sprouted plants were grown as described by Islam and Kawasaki (2014). Plants with three expanded leaf blades were subjected to one of the four treatments; 0 mM calcium, 1 mM calcium nitrate [Ca(NO$_3$)$_2$] (control), 15 mM calcium nitrate [Ca(NO$_3$)$_2$] and 15 mM calcium chloride (CaCl$_2$) in water culture solution for 7 d in a growth chamber (Islam and Kawasaki, 2014). After the treatment, plants were sampled and used for subsequent investigations.

2. **Measurement of leaf blade, petiole and whole-plant calcium contents**

    Calcium contents of leaf blades and petioles were measured using a flame atomic absorption spectrophotometer as reported previously (Islam and Kawasaki, 2014).
Kawasaki, 2014). Whole-plant calcium content was calculated by summing total calcium contents of leaf blades, petioles, corms and roots of the plant. Six plants per treatment were used for calcium determination.

3. Measurement of volume and calcium content of guttation-fluid

In eddo, guttation takes place in darkness from the evening until dawn. Guttation fluid exuded from leaf blade tips and margins was collected in conical flasks equipped with funnels each morning during 3 – 7 d after starting the treatment. The volume of fluid collected nightly was measured with a graduated cylinder. Guttation fluid was diluted with distilled water and filtered through cellulose membrane syringe filter (0.45 µm pore size). Calcium content was analyzed by flame atomic absorption spectrophotometry. Guttation fluid exuded from one complete leaf blade was recorded for each plant, and five plants measured per treatment.

Additionally, the ratio of calcium content of guttation fluid collected nightly per leaf blade to that of leaf blades in a plant, leaves (leaf blades and petioles) in a plant and a whole plant were calculated.

4. Bright-field optical microscopy and measurement of the number and size of the crystals

Center portions of leaf blades and middle sections of petioles were sampled and immersed in a formalin-acetic acid-alcohol solution at 20°C under reduced pressure conditions for 1 hr and then immersed in the solution at 20°C for 3 d. The leaf blade segments were hydrated in an ethanol series, rinsed with distilled water and embedded in agar. Cross sections (100 µm thick) were made with a microslicer (DTK-1000; D.S.K.). The sections were placed on glass slides, covered with cover slides and were observed under a bright-field optical microscope (BX51; Olympus). The number and size of raphide crystals were investigated using 4.39-mm-long cross sections of leaf blades having similar thicknesses. To measure the size and number of crystals in petioles, we used a sectioning method different from that used for leaf blades. Though this sectioning was more effective for measuring crystal number and size in petioles, it could not be easily applied to measure crystal number and size in leaf blades with the thin sections. The petiole segments were dehydrated in an ethanol series, immersed in t-butyl alcohol and embedded in paraffin. Longitudinal sections (10 µm thick) were generated with a microtome (RV-240; YAMATO), and then stained and observed under a bright-field optical microscope (Islam and Kawasaki, 2014). The number and size of raphide and druse crystals in 15.84 mm² (3.33 mm long × 4.8 mm wide) areas of the petiole longitudinal sections were investigated. Crystal sizes were calculated by measuring their areas using Image J software. One leaf blade and one petiole were examined per plant, with five leaf blades and five petioles investigated per treatment. To compare the number and size of crystals in leaf blades and petioles among treatments, we investigated and statistically analyzed two sections of each observed leaf blade and petiole.

5. Scanning electron microscopy and calcium mapping

Central portions of leaf blades and middle sections of petioles were sampled, frozen rapidly in liquid nitrogen and freeze-dried under a pressure of 11 Pa at ~20°C for 50 hr using a vacuum freeze dryer (FDU-1200; EYELA). The dried sections of leaf blades and petioles were cracked transversely and longitudinally, respectively. The 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 (SEM, JSM-7000F; JEOL) at an accelerating voltage of 5 kV to identify the structure of calcium oxalate crystals. Calcium localization on the cracked leaf blade and petiole sections was investigated using a SEM coupled to an energy dispersive X-ray spectrometer (JED-2300F; JEOL) at an accelerating voltage of 20 kV. One leaf blade and one petiole per plant, five leaf blades and five petioles per treatment were used.

6. Measurement of calcium weight percentage

The samples prepared for SEM observation and the calcium mapping were also used for this measurement. The weight percentage of calcium relative to the total weight of major constituent elements (Islam and Kawasaki, 2014) per mesophyll cell (spongy cell and palisade cell) and per crystal idioblast of leaf-blade cross sections was measured using a SEM attached to an energy dispersive X-ray spectrometer at an accelerating voltage of 20 kV. In this study, mesophyll cells refer to the spongy and palisade cells excluding crystal idioblasts. We also measured the percentage of calcium per normal parenchyma cell of ground tissue and per crystal idioblast of the petiole longitudinal sections. Analysis areas for this measurement were about 65 µm² (10.4 µm long × 6.25 µm wide) for the leaf-blade mesophyll cells and idioblasts and about 248 µm² (20 µm long × 12.4 µm wide) for the normal parenchyma cells and idioblasts in the petiole. The area analyzed in idioblasts encompassed the crystal as well as the cytosol. One leaf blade and one petiole per plant, five leaf blades and five petioles per treatment were investigated. Two mesophyll cells (one spongy cell and one palisade cell) near the crystal idioblast and two crystal idioblasts were measured per leaf blade section. In addition, two crystal idioblasts and two normal parenchyma cells near the crystal idioblast were measured per petiole section.
7. Statistical analysis

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

Results

1. Calcium contents of leaf blades, petioles and whole plants

Calcium contents (mg g⁻¹ dry weight and mg plant⁻¹) of leaf blades, petioles and whole plants subjected to 1 mM Ca(NO₃)₂ treatment were significantly (P < 0.01) higher than those subjected to 0 mM calcium treatment but significantly (P < 0.01) lower than those subjected to 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Fig. 1). No significant difference was observed in leaf blade, petiole and whole-plant calcium contents between the 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Fig. 1).

2. Guttation fluid amount and calcium contents

The amount of guttation fluid collected from 0 mM calcium and 1 mM Ca(NO₃)₂ treatments was significantly (P < 0.01) higher than that obtained from 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Fig. 2). No significant difference was observed in the amount of guttation fluid between the 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Fig. 2). Calcium contents (µg mL⁻¹) of guttation fluid under 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments were significantly (P < 0.01) higher than those obtained from 0 mM calcium and 1 mM Ca(NO₃)₂ treatments (Fig. 3A). Although the calcium content of guttation fluid from the 1 mM Ca(NO₃)₂ treatment was slightly higher than that from the 0 mM calcium treatment, the difference was not statistically significant (Fig. 3A). No significant difference was found in calcium contents of guttation fluid between the 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Fig. 3A).
Total calcium content (µg leaf⁻¹ night⁻¹) of guttation fluid was highest under 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments, followed by that obtained under 1 mM Ca(NO₃)₂ and 0 mM calcium treatments (Fig. 3B). The differences were significant (P < 0.01) statistically (Fig. 3B). No significant difference in total calcium content of guttation fluid was observed between the 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Fig. 3B).

The ratio of calcium content of guttation fluid collected nightly per leaf with respect to the calcium content of leaf blades, leaf blades plus petioles, and the entire plant was slightly higher under 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments than those under 1 mM Ca(NO₃)₂ and 0 mM calcium treatments (Table 1). These differences were not significant statistically.

3. Distribution and structure of calcium oxalate crystals

Under optical microscopy, raphide crystals were observed in leaf blade sections (Fig. 4), where they were present in spongy tissues and near the spongy-palisade tissue boundary. In petioles, raphide and druse crystals were observed in normal parenchyma cells of ground tissues (Fig. 4). Crystals in leaf blade and petiole sections of plants subjected to 1 mM Ca(NO₃)₂ treatment were smaller and less numerous than those of plants subjected to 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments. The fewest and smallest crystals were detected in sections of plants subjected to the 0 mM calcium treatment (Fig. 4). Under scanning electron microscopy, both raphide and druse crystals were observed in leaf blades (Fig. 5). Similar to the location of the raphide crystals, the druse crystals were present in leaf blades in spongy tissues and in the lower palisade tissue region. As the druses in leaf blades were smaller than those in the petioles they could not be observed by optical microscopy: 0.35 vs. 2.31 µm in diameter, under 1 mM Ca(NO₃)₂ treatment. The detected raphide crystals in leaf blades and petioles were present as bundles of needles in the idioblasts (Fig. 5). In leaf blades, druse crystals in the idioblasts were platy and tubular in form, whereas those in petioles were tetrahedral form (Fig. 5). Crystals in the leaf blades and petioles appeared to increase in size with increasing calcium concentration in the treatment solution (Fig. 5).

4. Crystal number and size

The number and size of calcium oxalate crystals were investigated using optical microscopic images of leaf blades and petioles. The number and size of raphide crystals in leaf blades subjected to 1 mM Ca(NO₃)₂ treatment were significantly (P < 0.01) greater than those subjected to 0 mM calcium treatment, but were significantly (P < 0.01)
smaller than those subjected to 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Figs. 6A and B). No significant difference was detected in the number and size of crystals in the leaf blades between the 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Figs. 6A and B). Druse crystals were not visible in leaf blades under optical microscopy and could not be accurately investigated on the cracked surface of samples prepared for SEM observation. In petioles, the number and size of the raphide and druse crystals observed in 1 mM Ca(NO₃)₂ treatment were significantly (P < 0.01) larger than that observed in 0 mM calcium treatment. In contrast, they were significantly (P < 0.01) smaller than those observed in 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Figs. 6C, D, E and F). There were no significant differences in the number and size of crystals in petioles between the 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments (Figs. 6C, D, E and F).

5. Calcium localization

SEM images and energy dispersive X-ray spectrometric calcium mapping images of cracked sections of leaf blades and petioles are shown in Fig. 7. Red dots indicate the presence of calcium. In crystal idioblasts, the intensity of dots in leaf blades and petioles was highest in plants subjected to 15 mM Ca(NO₃)₂ and 15 mM CaCl₂ treatments, followed by those subjected to 1 mM Ca(NO₃)₂ and 0 mM calcium treatments (Fig. 7). The pattern of calcium distribution in palisade and spongy mesophyll cells of leaf blades and normal parenchyma cells in ground tissues of petioles was nearly uniform in all treatments (Fig. 7).

6. Weight percentage of calcium per cell in the leaf blades and petioles

The weight percentage of calcium per mesophyll cell and per crystal idioblast of leaf blades did not vary significantly among the treatments (Fig. 8A). However, the weight percentage of calcium per crystal idioblast was
significantly ($P < 0.001$) higher than that per mesophyll cell across the treatments (Fig. 8A). Similarly, the weight percentage of calcium per normal parenchyma cell and per crystal idioblast of petioles did not vary significantly among the treatments (Fig. 8B). The weight percentage of calcium per crystal idioblast was also significantly ($P < 0.001$) higher than that per normal parenchyma cell of petioles in all treatments (Fig. 8B).

**Discussion**

Calcium contents (mg g$^{-1}$ dry weight and mg plant$^{-1}$) of leaf blades, petioles and whole plants increased with increasing calcium concentration in the culture solutions (Fig. 1). These results indicate that the experimental treatments were able to alter plant calcium content. A positive correlation has been found between calcium concentrations in growth medium and the number and size of calcium oxalate crystals in leaves of *Pistia stratiotes* (Volk et al., 2002), *Phaseolus vulgaris* (Kuo-Huang and Zindler-Frank, 1998), *Lemna minor* (Mazen et al., 2004), *Corchorus olitorius* (Faheed et al., 2013) and *Malva parviflora* (Faheed et al., 2013). In a study of eddo roots subjected to different calcium concentrations, the number and size of crystals in the root apical zone under 1 mM Ca(NO$_3$)$_2$ solution were significantly larger than those in 0 mM calcium solution and smaller than those in 15 mM calcium solutions (Islam and Kawasaki, 2014). In this study, a similar result was observed in leaf blades and petioles of eddo with respect to different calcium concentrations (Fig. 6), demonstrating that the properties of crystals in eddo leaves are dependent on the calcium concentration of the growth medium.

In this study, calcium mapping images revealed that the calcium was most abundant in crystal idioblasts of leaf blades and petioles of plants subjected to the most concentrated calcium treatments (Fig. 7). However, the pattern of calcium distribution in leaf-blade mesophyll cells and normal parenchyma cells of petioles was nearly uniform across the treatments (Fig. 7). In our earlier study involving the apical zone having calcium oxalate crystals of eddo roots (Islam and Kawasaki, 2014), the weight percentage of calcium relative to the total weight of major constituent elements of cortical parenchyma cells did not vary significantly among different calcium concentrations in the growth medium. In the root zone apart from the root apex having no crystals, the weight percentage of calcium per cortical parenchyma cell was significantly higher in solutions containing 15mM calcium than in either 0 mM calcium or 1 mM Ca(NO$_3$)$_2$ solution. In the present study, 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 (Fig. 8). On the other hand, the weight percentage of calcium per mesophyll cell...
of leaf blades and per normal parenchyma cell of petioles was stable among the treatments (Fig. 8). 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 stabilization of calcium levels in leaf tissues other than the idioblasts.

Guttation is a common phenomenon in cereal leaves and may serve as a salt-loss pathway in those species (Tester and Davenport, 2003). Shapira et al. (2013) have reported that guttation is involved in boron homeostasis in the laminae of banana. In our study, the calcium content of guttation fluid was significantly increased when the calcium concentration of the culture solution was raised (Fig. 3). In some plants of Colocasia species in tropical areas, a single leaf may lose over 300 mL of water in one night through guttation (Moore et al., 2003). In our study, an average of 6.36 mL of guttation fluid was exuded nightly from a single eddo leaf in the control [1 mM Ca(NO₃)₂] group (Fig. 2). Although the exuded volume of guttation fluid was significantly lower in leaves of plants subjected to 15 mM calcium treatments (Fig. 2), the total amount of calcium excreted nightly per leaf was significantly larger than that in the control group (Fig.
The calcium contents of leaf blades, petioles and whole plants were increased with the increase in the calcium concentration in the growth medium (Fig. 1). Nevertheless, the ratio of calcium content of guttation fluid collected nightly from a single leaf blade to that of leaf blades, leaf blades plus petioles and whole plant was slightly increased with increasing calcium concentration in the treatment solution (Table 1). As described above, the weight percentage of calcium per mesophyll cell of leaf blades and per normal parenchyma cell of petioles were stable among the treatments (Fig. 8). These results suggest that guttation excludes excess calcium and would be involved in maintaining calcium homeostasis in eddo plants.

Crystal-containing raw forage materials are irritants to workers during handling (Carpenter and Steinke, 1983). Consumption of raw or partially processed plant materials containing calcium oxalate crystals causes a burning, itching sensation in the throat and mouth epithelium and indirectly affects feed intake (Bradbury and Nixon, 1998). Calcium oxalate is an insoluble biomineral and a component of ash that is directly related to ash yield (Erdman et al., 1977). Calcium oxalate crystals may lower the nutritive value of forage, with excessive ash content of forage thus possibly acting as a silent antagonist in dairy nutrition program performance. Calcium is a major component of calcium oxalate crystals. If the role of guttation in the elimination of calcium can be controlled, the regulation of crystal formation and calcium tolerance in crop plants will be possible.

Our investigation is the first to associate calcium regulation with calcium oxalate crystals in leaves and guttation of eddo grown under different calcium concentrations. The knowledge generated by our study should contribute to elucidation of the overall mechanism of calcium regulation in eddo, thereby leading to improvements in growth, tolerance against calcium stress and eating quality of leaf blades and petioles.

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.
Fig. 8. Weight percentage of calcium per cell of leaf blades and petioles.

The weight percentage of calcium per mesophyll cell and per crystal idioblast of leaf blades (A) and per normal parenchyma cell and per crystal idioblast of petioles (B) was measured by energy dispersive X-ray spectrometry. Different letters indicate significant differences at the 0.1% level (n = 10, Tukey’s test).

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