Effect of Calcium Concentration in Growth Medium on Oxalate Content and Evaluation of the Role of Guttation in the Regulation of Oxalate in Eddo

Mohammad Nazrul Islam¹, Hayato Maeda¹,² and Michio Kawasaki¹,²

(¹The United Graduate School of Agricultural Sciences, Iwate University, 3-18-8 Ueda, Morioka 020-8550, Japan; ²Faculty of Agriculture and Life Sciences, Hirosaki University, 3 Bunkyo-cho, Hirosaki 036-8561, Japan)

Abstract: The effects of calcium concentrations in the growth medium on oxalate content of leaf blades, petioles and corms and the involvement of guttation in the regulation of oxalate homeostasis were investigated in eddo (Colocasia esculenta (L.) Schott var. antiquorum Hubbard & Rehder). The plants were grown hydroponically in solutions containing 0 mM calcium, 1 mM calcium nitrate (control), 15 mM calcium nitrate or 15 mM calcium chloride. Total oxalate content (soluble plus insoluble) of leaf blades, petioles and corms did not differ with the calcium concentration in solutions containing 1 mM or 15 mM calcium nitrate or 0 mM calcium. The soluble oxalate content of these parts decreased as the calcium concentration of the solution was increased. Solutions containing 15 mM calcium nitrate or 15 mM calcium chloride gave a significantly lower proportion of soluble oxalate content to total oxalate content in each part, especially in leaf blades than 0 mM calcium or 1 mM calcium nitrate. In contrast, a positive correlation was found between insoluble oxalate content and calcium concentration in the solution. These results demonstrate that high calcium concentrations in the growth medium reduce soluble oxalate content of the plant. Soluble oxalate was detected in eddo guttation fluid. Soluble oxalate content in this fluid (mg mL⁻¹) and the amount of soluble oxalate exuded by guttation (mg leaf⁻¹ night⁻¹) were significantly lower in the solutions containing 15 mM calcium than in those containing 0 mM and 1 mM calcium. These results indicate that guttation may affect the concentration of soluble oxalate in the plant bodies although not strongly contributing to a decrease in soluble oxalate content in eddo grown under high calcium conditions.

Key words: Calcium, Colocasia esculenta, Eddo, Guttation, Oxalate.

Eddo (Colocasia esculenta (L.) Schott var. antiquorum Hubbard & Rehder), an important food crop in the taro (Colocasia esculenta) group, is mainly cultivated in Asia. The corms of taro are highly valued as a staple food for human consumption. Additionally, taro leaf blades and petioles, including those abundantly produced in eddo, are used as animal feed in countries such as Laos, Cambodia (Buntha et al., 2008) and Vietnam (Hang et al., 2011). The leaf blades, petioles and corms of most taro cultivars contain high oxalate levels (Oscarsson and Savage, 2007; Catherwood et al., 2007; Hang et al., 2013). Because they can form non-absorbable insoluble salts with Ca²⁺, Fe²⁺ and Mg²⁺, thereby rendering these minerals unavailable, oxalates are anti-nutritive compounds (Savage et al., 2000, 2009; Quinteros et al., 2003; Oscarsson and Savage, 2007). Depending upon plant species, oxalate primarily accumulates either as soluble oxalate, insoluble oxalate or a combination of these two forms (Libert and Franceschi, 1987). A diet high in soluble oxalate is known to lead to excessive urinary excretion of oxalate (hyperoxaluria) and consequently an increased risk of kidney stone development (Holmes and Assimos, 2004). Various post-harvest processing methods for the reduction of oxalate content of taro and its products have been developed (Oscarsson and Savage, 2007; Catherwood et al., 2007; Savage et al., 2009; Hang and Preston, 2010; Hang et al., 2013), but information on the agronomic aspects of regulating oxalate contents in taros is still limited.

The number and size of calcium oxalate crystals have been reported to be positively correlated with the amount of calcium supplied in the growth medium (Kuo-Huang and Zindler-Frank, 1998; Volk et al., 2002; Mazen et al., 2004; Faheed et al., 2013). For example, a positive correlation has been observed between calcium...
concentration in the growth medium and calcium oxalate crystals in the apical zone of primary roots of eddo (Islam and Kawasaki, 2014), and in leaf blades and petioles of eddo (Islam and Kawasaki, 2015). While some researchers have focused on the effect of growth-medium calcium on soluble oxalate content, information on the relationship between calcium in the growth medium and soluble oxalate in plant bodies is limited. In Phaseolus vulgaris, total oxalate content of growing leaves was found to be independent of calcium supply. Soluble oxalate was higher in leaves of the plants grown in nutrient solution with a low calcium concentration, whereas insoluble oxalate was higher in leaves of the plants grown in a solution with a high calcium concentration (Zindler-Frank et al., 2001). In spinach leaves, an increase in calcium concentration has been observed to reduce soluble oxalate content (Zhang et al., 2009). Rahman et al. (2009) reported that soluble oxalate levels in Napier grass (Pennisetum purpureum Schumach.) can be partially reduced by using calcium hydroxide. To our knowledge, the effect of calcium concentration in the growth medium on the soluble oxalate contents of eddo has not been determined.

Guttation is the process of liquid exudation from hydathodes situated at the tips, margins and adaxial and abaxial surfaces of leaves in some plants (Singh, 2014). Guttation fluids contain various salts, amino acids, proteins and sugars (Curtis, 1943; Ivanoff, 1963; Goatley 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 salts are said to be exuded from hydathodes via their dissolution in the guttation fluid (Tester and Davenport, 2003), however, experimental demonstrations in relation to the role of guttation in the maintenance of homeostasis in plant bodies is limited. In taros, large volumes of guttation fluid are exuded from leaf blade hydathodes (Moore et al., 2003). However, the role of guttation in regulating the oxalate homeostasis in taros, including eddo, is not understood.

Methods to reduce plant soluble oxalate concentrations must be investigated to develop strategies for improving the nutritional value of eddo as food and forage. In this study, we thus aimed to show the effects of growth-medium calcium concentration on oxalate content of eddo leaf blades, petioles and corms and to investigate the role of guttation in oxalate homeostasis of eddo grown hydroponically under different calcium levels.

Materials and Methods

1. Plant materials and treatment solutions

Plants of eddo cultivar Aichiwase were used in this study. Seed corms were planted in plastic pots filled with vermiculite in August 2013, and the sprouted plants were grown as described by Islam and Kawasaki (2014). Plants with three expanded leaf blades were grown hydroponically in a treatment containing 0 mM calcium, 1 mM calcium nitrate, which was the normal concentration, 15 mM calcium nitrate or 15 mM calcium chloride for 7 days. The calcium chloride treatment helps us to evaluate the effect of calcium. To maintain nitrate levels identical to those in the solution containing 15 mM calcium nitrate, we added ammonium nitrate to the other three solutions. Plants cultured in these solutions showed no abnormal growth, stress disorders or reproductive growth transition (such as flower-bud formation). After treatment, plants were sampled and examined for oxalate contents. Guttation fluid was obtained using plants cultured in the four solutions for designated periods.

2. Measurement of leaf blade, petiole and corm oxalate contents

Leaf blades, petioles and corms of eddo were sampled separately and stored immediately at −20°C. Samples were freeze-dried for 3 days using a vacuum freeze-drying system (DFR-05N-B; ULVAC, Kanagawa), ground to a fine powder in a mortar with a pestle and stored in a desiccator. Total and soluble oxalate contents of 0.5 g of each finely ground sample were determined by high performance liquid chromatography (HPLC) as reported by Savage et al. (2000). The samples were put into 125-mL beakers and 25 mL of 2 M hydrochloric acid was then added. The beakers were placed in a water bath at 80°C for 15 min. After being cooled, the extract was transferred to a 50-mL volumetric flask and 2 M hydrochloric acid was filled to the marked line. Soluble oxalate content was measured using distilled water instead of hydrochloric acid. The extracts were centrifuged at 3,000 rpm (815 g) (MX-305; Tomy Seiko, Tokyo) for 15 min, and 5 mL of the supernatant was then filtered through a 0.45-μm cellulose membrane syringe filter (Starlab Scientific). Chromatographic separation was carried out on a 250 × 4.6 mm ion exclusion column (Inertsil ODS-4; GL Sciences, Tokyo) using a Hitachi HPLC system composed of an L-7100 pump, an L-7420 UV/VIS detector set to a wavelength of 210 nm. Data capture and processing were conducted using the Hitachi D-7000 HPLC system manager. For separation, we used an aqueous solution of 25 mM sulfuric acid as the mobile phase. Samples (2 μL) were injected onto the column and eluted at a flow rate of 0.5 mL min⁻¹. The oxalate peaks were identified based on the retention time of the standard solution of oxalate alone and that of the oxalate-spiked unknown samples. Oxalate concentrations were calculated using standard calibration curves prepared from oxalic acid (Wako Pure Chemical). Insoluble oxalate content was calculated by subtracting soluble oxalate content from total oxalate content (Holloway et al., 1989).

Plant materials and treatment solutions

Materials and Methods
3. Measurement of guttation fluid volumes and the fluid soluble oxalate contents

In eddo, guttation takes place in darkness from evening until dawn. Guttation fluid exuded from the leaf blades was collected in conical flasks equipped with funnels each morning from day 3 to 7 of culture in the solution containing calcium as described above. The volume of fluid collected nightly was measured with a graduated cylinder. The guttation fluid was filtered through a 0.45-μm cellulose membrane syringe filter and chromatographically separated as described above. Samples (5 μL) were applied to the column and the oxalate concentrations were measured as described above. Guttation fluid exuded from one complete leaf blade was recorded for each plant, five plants per treatment solution.

4. Statistical analysis

Analysis of variance was followed by Tukey’s test for data on the oxalate content obtained from eddo guttation fluid, leaf blades, petioles and corms.

Results

1. Total oxalate contents

In leaf blades, no significant differences were found in total oxalate content among 0 mM calcium, and 1 mM and 15 mM calcium nitrate solutions (Fig. 1A). In contrast, 15 mM calcium chloride treatment gave significantly (P < 0.05) lower oxalate contents of leaf blades than 0 mM calcium and 1 mM calcium nitrate solutions (Fig. 1A). Oxalate contents of leaf blades were not significantly different between the 15 mM calcium solutions. Total oxalate contents of petioles, which did not vary significantly with the solution, were markedly higher than those of leaf blades and corms at all growth-medium calcium concentrations (Fig. 1B). In corms, no significant differences were observed in total oxalate content among 0 mM
calcium and 1 mM and 15 mM calcium nitrate solutions (Fig. 1C), whereas the 15 mM calcium chloride solution gave significantly \( (P < 0.05) \) lower oxalate contents of corms than 0 mM calcium or 1 mM or 15 mM calcium nitrate solutions.

2. **Soluble oxalate contents**

   Soluble oxalate contents of leaf blades, petioles and corms in 15 mM calcium chloride and 15 mM calcium nitrate solutions were significantly \( (P < 0.05) \) lower than those in 0 mM calcium and 1 mM calcium nitrate solutions (Fig. 2). In leaf blades, soluble oxalate content in 1 mM calcium nitrate solution was significantly \( (P < 0.05) \) lower than that in 0 mM calcium solution (Fig. 2A). In corms, soluble oxalate content was not significantly different between 0 mM calcium and 1 mM calcium nitrate solutions (Fig. 2C). Similarly, contents of leaf blades, petioles and corms in the solution containing 15 mM calcium nitrate did not significantly differ from that containing 15 mM calcium chloride. The soluble oxalate content of petioles was also higher than that of leaf blades and corms in each solution.

3. **Insoluble oxalate contents**

   Figure 3 shows insoluble oxalate contents of leaf blades, petioles and corms. In all three plant parts, insoluble oxalate content was significantly \( (P < 0.05) \) higher in the 15 mM calcium nitrate and 15 mM calcium chloride solutions than in the 0 mM calcium and 1 mM calcium nitrate solutions (Fig. 3). Insoluble oxalate contents of leaf blades and petioles in the 1 mM calcium nitrate solution were also significantly \( (P < 0.05) \) higher than those in the 0 mM calcium treatment (Figs. 3A, B). In contrast, no significant difference was observed in any plant parts between the 15 mM calcium nitrate and 15 mM calcium chloride solutions. Insoluble oxalate was also more abundant in petioles than in leaf blades and corms.

4. **Soluble oxalate content-total oxalate content ratios**

   In all plant parts, the ratios of soluble oxalate content to total oxalate content were significantly \( (P < 0.05) \) lower in the 15 mM calcium nitrate and 15 mM calcium chloride solutions than in the 0 mM calcium and 1 mM calcium nitrate solutions (Table 1). In leaf blades and petioles, ratios were also significantly \( (P < 0.05) \) lower in the 1 mM calcium nitrate solution than in the 0 mM calcium solution (Table 1). However, no significant differences between the

---

| Treatments      | Ratios of soluble oxalate contents (%) |
|-----------------|----------------------------------------|
|                 | Leaf blades | Petioles | Corms       |
| 0 mM Ca         | 82.28 ± 2.10 a | 74.67 ± 3.61 a | 88.85 ± 3.70 a |
| 1 mM Ca(NO\(_3\))\(_2\) (Control) | 59.98 ± 8.09 b | 62.72 ± 4.86 b | 82.55 ± 6.86 a |
| 15 mM Ca(NO\(_3\))\(_2\) | 27.00 ± 5.56 c | 47.61 ± 4.49 c | 62.59 ± 5.32 b |
| 15 mM CaCl\(_2\) | 20.04 ± 4.89 c | 44.40 ± 2.42 c | 59.83 ± 5.46 b |

Values are represented as means ± SD \( (n = 5) \). Different letters within the same column indicate significant differences among treatments at the 5% level (Tukey's test).
15 mM calcium nitrate and 15 mM calcium chloride solutions were found in these ratios in leaf blades, petioles and corms. Compared with the results obtained in the 1 mM calcium solution, the ratios in leaf blades, petioles and corms were 32.98, 15.11 and 19.96 points lower, respectively, in the 15 mM calcium nitrate solution, with corresponding decreases of 39.94, 18.92 and 22.72 points in the 15 mM chloride nitrate solution.

5. Guttation fluid volumes, soluble oxalate contents of the fluid and the amount of soluble oxalate exuded by guttation

The volume of guttation fluid collected from the plants in the 0 mM calcium and 1 mM calcium nitrate solutions was significantly ($P < 0.05$) higher than that obtained in either the 15 mM calcium nitrate or 15 mM calcium chloride solution (Fig. 4A). There were no significant differences in the guttation fluid volumes between the 0 mM calcium and 1 mM calcium nitrate solutions nor between the 15 mM calcium nitrate and 15 mM calcium chloride solutions.

The oxalate exuded by guttation was detected as soluble oxalate. The soluble oxalate content of guttation fluid (mg mL$^{-1}$) and the amount of soluble oxalate exuded by guttation (mg leaf$^{-1}$ night$^{-1}$) in either 15 mM calcium nitrate or 15 mM calcium chloride solution were significantly ($P < 0.05$) lower than that in the 1 mM calcium nitrate and 0 mM calcium solutions (Fig. 4B, C). In contrast, no significant differences in soluble oxalate contents of guttation fluid and the amount of soluble oxalate exuded by guttation were recorded between 0 mM calcium and 1 mM calcium nitrate solutions or between 15 mM calcium nitrate and 15 mM calcium chloride solutions.

6. Levels of soluble oxalate in guttation fluid vs. total oxalate in leaf blades, petioles and corms

The ratios of the soluble oxalate content of guttation fluid from one leaf per night (mg leaf$^{-1}$ night$^{-1}$) to the total oxalate content of all leaf blades, petioles and corms in one plant (mg plant$^{-1}$) are shown in Table 2 for each part and for the sum of the three parts in the entire plant. In leaf blades, corms and the sum of the three parts, ratios obtained in the 15 mM calcium solutions were lower than those obtained in the 0 mM or 1 mM calcium solutions. In petioles, the ratios obtained in the 15 mM calcium nitrate solutions were found in these ratios in leaf blades, petioles and corms. Compared with the results obtained in the 1 mM calcium solution, the ratios in leaf blades, petioles and corms were 32.98, 15.11 and 19.96 points lower, respectively, in the 15 mM calcium nitrate solution, with corresponding decreases of 39.94, 18.92 and 22.72 points in the 15 mM chloride nitrate solution.

**Table 2.** Ratio of the amount of soluble oxalate exuded by guttation (mg leaf$^{-1}$ night$^{-1}$) to total oxalate content of all leaf blades, petioles or corms in one plant (mg plant$^{-1}$) and to the sum of total oxalate content of these parts in one plant (mg plant$^{-1}$).

| Treatments | Leaf blades | Petioles | Corms | Leaf blades + Petioles + Corms |
|------------|-------------|----------|-------|-------------------------------|
| 0 mM Ca    | 5.58 ± 1.90 a | 1.38 ± 0.73 ac | 19.01 ± 4.55 a | 1.02 ± 0.48 a |
| 1 mM Ca(NO$_3$)$_2$ (Control) | 5.49 ± 1.02 a | 1.25 ± 0.42 cd | 18.76 ± 5.70 a | 0.95 ± 0.28 a |
| 15 mM Ca(NO$_3$)$_2$ | 2.13 ± 1.34 b | 0.41 ± 0.06 b | 5.45 ± 1.82 b | 0.31 ± 0.07 b |
| 15 mM CaCl$_2$ | 2.25 ± 0.93 b | 0.49 ± 0.26 bd | 5.91 ± 1.92 b | 0.37 ± 0.18 b |

Values are represented as means ± SD ($n = 5$). Different letters within the same column indicate significant differences among treatments at the 5% level (Tukey’s test).
and 15 mM calcium chloride solutions were lower than that obtained in the 0 mM calcium solution. Additionally, in petioles, the ratio obtained in the 15 mM calcium nitrate solution was lower than that obtained in the 1 mM calcium nitrate solution. The ratio was lower in petioles than in leaf blades and corms because petioles had higher oxalate contents than the leaf blades and corms.

**Discussion**

Oxalate is often considered an end product of metabolism. Oxalate is universally found in plant tissues, but its distribution in the plant is not homogeneous (Zhang et al., 2009). The oxalate content of leaves is generally higher than that of roots (Libert and Franceschi, 1987). Stems or stalks of plants have significantly lower oxalate contents than leaves (Noonan and Savage, 1999). In some taro cultivars grown in central Vietnam, total oxalate content has been found to be similar in leaf blades and petioles, with a tendency towards less soluble and more insoluble oxalate in leaf blades compared with petioles (Hang et al., 2011). Another study (Hang and Preston, 2010) of Vietnamese-grown taro has revealed higher levels of total oxalate in petioles than in leaf blades. In our study, soluble and insoluble oxalate contents were markedly higher in eddo petioles than in leaf blades and corms (Figs. 1, 2 and 3).

Soluble oxalate content of eddo leaf blades, petioles and corms decreased (Fig. 2) and insoluble oxalate content increased with the increase in the calcium concentration in the solution (Fig. 3). Similar trends have been reported in *Phaseolus vulgaris* and Napier grass (Zindler-Frank et al., 2001; Rahman et al., 2009). We also found that total oxalate content of eddo leaf blades, petioles and corms did not differ significantly with the solution (Fig. 1). The amounts of calcium oxalate crystals in eddo leaf blades, petioles and roots were previously found to increase in growth medium containing high calcium concentrations (Islam and Kawasaki, 2014, 2015). Consequently, the combination of excess calcium with soluble oxalate to form insoluble oxalates, i.e., oxalate calcium crystals, under high calcium conditions would lower the soluble oxalate content. The lower total oxalate content of leaf blades and corms in the 15 mM calcium chloride solution (Fig. 1) may be due to the effect of chloride from calcium chloride. Singh (1974) reported that chloride and other anions compete for cations and depress oxalate synthesis. Low levels of both total and soluble oxalates in saltbush leaves have been found to be accompanied by high levels of chlorine (Ellern et al., 1974). These findings imply that high calcium concentrations in the growth medium reduced soluble oxalate content and increased insoluble oxalate content of the eddo leaf blades, petioles and corms in our study. Average ratios of soluble to total oxalate contents in both 15 mM calcium nitrate and 15 mM calcium chloride solutions were lowered by 32.46 points in leaf blades, 16.72 points in petioles and 21.34 points in corms as compared to those in the 1 mM calcium nitrate solution (Table 1). This result indicates that soluble oxalate in eddo leaf blades is more susceptible to the effect of calcium application than that in petioles and corms. In addition, our results suggest that adjustment of the calcium fertilizer application rate is a potentially effective agronomic practice for regulating the soluble oxalate content of eddo.

In this study, we confirmed the presence of soluble oxalate in eddo guttation fluid. The soluble oxalate content (mg mL⁻¹) of guttation fluid and the amount of soluble oxalate exuded by guttation (mg leaf⁻¹ night⁻¹) were significantly reduced in the plants grown in a culture solution containing high calcium concentrations (Fig. 4B, C). Additionally, a higher calcium concentration in the solution lowered the ratios of the amount of soluble oxalate exuded by guttation (mg leaf⁻¹ night⁻¹) to the total oxalate content of leaf blades, corms or combined leaf blades, petioles and corms (Table 2). Therefore, guttation exudes soluble oxalate and may affect its concentration in the plant. However, this exudation would not strongly contribute to a decrease in soluble oxalate content of eddo grown under high calcium conditions. The present findings contribute valuable information on the regulation of soluble oxalate levels in eddo and consequently will lead to improvement in the eating quality of eddo as an animal feed and as a human food staple.

**References**

Buntha, P., Borin, K., Preston, T.R. and Ogle, B. 2008. Digestibility and nitrogen balance studies in pigs fed diets with ensiled taro (*Colocasia esculenta*) leaves as replacement for fish meal. *Livest. Res. Rural Dev.* 20 (supplement).

Catherwood, D.J., Savage, G.P., Mason, S.M., Scheffer, J.J.C. and Douglas, J.A. 2007. Oxalate content of cormels of Japanese taro (*Colocasia esculenta* L.) Schott and the effect of cooking. *J. Food Compos. Anal.* 20: 147-151.

Chen, C.C. and Chen, Y.R. 2007. Study on laminar hydathodes of *Ficus formosana* (Moraceae). III. Salt injury of guttation on hydathodes. *Bot. Stud.* 48: 215-226.

Curtis, L.C. 1943. Detrimental effects of guttated fluids on foliage. *Am. J. Bot.* 30: 778-782.

Curtis, J.D. and Lersten, N.R. 1974. Morphology, seasonal variation, and function of resin glands on buds and leaves of *Populus deltoides* (Salicaceae). *Am. J. Bot.* 61: 835-845.

Dieffenbach, H., Kramer, D. and Lüttsge, U. 1980. Release of guttation fluid from passive hydathodes of intact barley plants. I. Structural and cytological aspects. *Ann. Bot.* 45: 397-401.

Ellern, S.J., Samish, Y.B. and Lachower, D. 1974. Salt and oxalic acid content of leaves of the saltbush *Atriplex halimus* in the northern Negev. *J. Range Manage.* 27: 267-271.

Faheed, F., Mazen, A. and Elmohsen, S.A. 2013. Physiological and ultrastructural studies on calcium oxalate crystal formation in...
some plants. *Tark. J. Bot.* 37: 139-152.

Goatley, J.L. and Lewis, R.W. 1966. Composition of guttation fluid from rye, wheat, and barley seedling. *Plant Physiol.* 41: 373-375.

Hang, D.T. and Preston, T.R. 2010. Effect of processing taro leaves on oxalate concentrations and using the ensiled leaves as a protein source in pig diets in central Vietnam. *Livest. Res. Rural Dev.* 22: 68.

Hang, D.T., Binh, L.V., Preston, T.R. and Savage, G.P. 2011. Oxalate concentration of different taro cultivars grown in central Viet Nam and the effect of simple processing methods on the oxalate concentration of the processed forages. *Livest. Res. Rural Dev.* 23: 122.

Hang, D.T., Vanhanen, L. and Savage, G. 2013. Effect of simple processing methods on oxalate content of taro petioles and leaves grown in central Viet Nam. *Food Sci. Technol.* 50: 259-263.

Holloway, W.D., Argill, M.E., Jealous, W.T., Lee, J.A. and Bradbury, J.H. 1989. Organic acids and calcium oxalate in tropical root crops. *J. Agric. Food Chem.* 37: 337-341.

Holmes, R.P. and Assimos, D.G. 2004. The Impact of dietary oxalate on kidney stone formation. *Urol. Res.* 32: 311-316.

Islam, M.N. and Kawasaki, M. 2014. Morphological changes and function of calcium oxalate crystals in eddo roots in hydronpic solution containing calcium at various concentrations. *Plant Prod. Sci.* 17: 13-19.

Islam, M.N. and Kawasaki, M. 2015. Evaluation of calcium regulating roles of guttation and calcium oxalate crystals in leaf blades and petioles of hydronically grown eddo. *Plant Prod. Sci.* 18: 11-21.

Ivanoff, S.S. 1963. Guttation injuries of plants. *Bot. Rev.* 29: 202-229.

Komarnytsky, S., Borisjuk, N.V., Borisjuk, L.G., Alam, M.Z. and Ivanoff, S.S. 1963. Guttation injuries of plants. *Bot. Rev.* 29: 202-229.

Kuo-Huang, L.L. and Zindler-Frank, E. 1998. Structure of crystal cells and influences of leaf development on crystal cell development and vice versa in *Phaseolus vulgaris* (Leguminosae). *Bot. Acta* 111: 337-345.

Libert, B. and Franceschi, V.R. 1987. Oxalate in crop plants. *J. Agric. Food Chem.* 35: 926-938.

Mazén, A.M.A., Zhang, D. and Franceschi, V.R. 2004. Calcium oxalate formation in *Lemna minor*: physiological and ultrastructural aspects of high capacity calcium sequestration. *New Phytol.* 161: 435-448.

Mizuno, N., Takahashi, A., Wagatsuma, T., Mizuno, T. and Obata, H. 2002. Chemical composition of guttation fluid and leaves of *Petasites japonicus* v. *giganteus* and *Polygonum cuspidatum* growing on ultramafic soil. *Soil Sci. Plant Nutr.* 48: 451-453.

Moore, R., Clark, W.D. and Vodopich, D.S. 2003. Botany, 2nd edition. McGraw-Hill, Inc., New York. 496-520.

Noonan, S.C. and Savage, G.P. 1999. Oxalate content of foods and its effect on humans. *Asia Pac. J. Clin. Nutr.* 8: 64-74.

Oscarsson, K.V. and Savage, G.P. 2007. Composition and availability of soluble and insoluble oxalates in raw and cooked taro (*Colocasia esculenta* var. Schott) leaves. *Food Chem.* 101: 559-562.

Pilot, G., Stransky, H., Bushey, D.F., Pratelli, R., Ludewig, U., Wingate, V.P.M. and Frommer, W.B. 2004. Overexpression of GLUTAMINE DUMPER1 leads to hypersecretion of glutamine from hydathodes of *Arabidopsis* leaves. *Plant Cell* 16: 1827-1840.

Quinteros, A., Farré, R. and Lagarda, M.J. 2003. Effect of cooking on oxalate content of pulses using an enzymatic procedure. *Int. J. Food Sci. Nutr.* 54: 373-377.

Rahman, M.M., Ishii, Y., Nimi, M. and Kawamura, O. 2009. Changes of oxalate form in pot-grown napiergrass (*Pennisetum purpureum Schumach.*) by application of calcium hydroxide. *Grassl. Sci.* 55: 18-22.

Savage, G.P., Vanhanen, L., Mason, S.M. and Ross, A.B. 2000. Effect of cooking on the soluble and insoluble content of some New Zealand foods. *J. Food Compos. Anal.* 13: 201-206.

Savage, G.P., Märtensson, L. and Sedcole, J.R. 2009. Composition of oxalate in baked taro (*Colocasia esculenta* var. Schott) leaves cooked alone or with addition of cows milk or coconut milk. *J. Food Compos. Anal.* 22: 83-86.

Shapira, O., Israeli, Y., Shani, U. and Schwartz, A. 2013. Salt stress aggravates boron toxicity symptoms in banana leaves by impairing guttation. *Plant Cell Environ.* 36: 275-287.

Singh, S. 2014. Guttation: Quantification, microbiology and implications for phytopathology. In U. Lütge, W. Beyschlag and J. Cushman eds., Progress in Botany, Volume 75. Springer-Verlag, Berlin/Heidelberg, 187-214.

Singh, P.P. 1974. Influence of light intensity, fertilizers and salinity on oxalate and mineral concentration of two vegetables (*Chenopodium album* L. and *Chenopodium amaranthicolor* L.). *Plant Foods Hum. Nutr.* 24: 115-125.

Tester, M. and Davenport, R. 2003. Na+ tolerance and Na+ transport in higher plants. *Ann. Bot.* 91: 505-527.

Volk, G.M., Lynch-Holm, V.J., Kostman, T.A., Goss, L.J. and Franceschi, V.R. 2002. The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in *Pistia stratiotes* leaves. *Plant Biol.* 4: 34-45.

Zhang, Y., Li, Y., Wei, J., Sun, M., Tian, Y. and Li, Z. 2009. Effects of nitrogen and calcium nutrition on oxalate contents, forms, and distribution in spinach. *J. Plant Nutr.* 32: 2123-2139.