Hydathode morphology and role of guttation in excreting sodium at different concentrations of sodium chloride in eddo

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Hydathode morphology and role of guttation in excreting sodium at different concentrations of sodium chloride in eddo

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ABSTRACT

Mature leaves of field-grown eddo plants were used for observing hydathode morphology by light and scanning electron microscopies. There were approximately five hydathode pores on the adaxial surface of each leaf tip. A ring structure with two borders around the pore was detected in this study. Further observations revealed a large cavity underneath the pores. The cavity was directly connected to vascular bundles that lacked a bundle sheath, via intercellular spaces among loosely organized parenchyma cells. Many crystal cells were present around the cavity and vascular bundles. To evaluate the role of guttation in sodium excretion under salinity stress, eddo plants were grown in hydroponic solutions containing 0, 1, 4, 8, and 12 mM sodium chloride (NaCl) for 7 d. As the NaCl concentration in the hydroponic solution increased, the sodium contents increased in leaf blades, petioles, and roots but remained unchanged in corms. The sodium concentration in the guttation fluid increased; however, the volume of guttation fluid decreased with increasing NaCl concentrations. Therefore, sodium elimination via guttation decreased with increasing NaCl concentrations. The ratios of the sodium content in guttation fluid to that of leaf blades, leaves, and whole plants decreased with increasing NaCl concentrations. The ratios of potassium to sodium contents in leaves, roots, and guttation fluid also decreased as the NaCl concentration increased. These results indicate that guttation did not eliminate sufficient sodium to play a role in adjusting sodium homeostasis and the ratios of potassium to sodium contents in eddo plants under saline conditions.

Eddo [*Colocasia esculenta* (L.) Schott var. *antiquorum* Hubbard & Rehder], a member of the taro group, is an important edible crop in the Araceae family. Guttation is a common phenomenon in taro (Arthur, 1920; Flood, 1919; Islam & Kawasaki, 2015; Müller, 1919). A single taro leaf is capable of excreting 5–300 mL of guttation fluid per night via the hydathodes under field conditions (Datta, 1994; Flood, 1919; Moore et al., 2003). Hydathode mediates guttation under high humidity and in the absence of transpiration at night. A few recent studies have attempted to describe hydathode morphology in some plant species. Hydathodes are located along the margin and on the adaxial or abaxial surfaces of leaves and consist of the pore, transfer or epithem cells, and a junction of xylem vessels or xylem endings beneath the pore (Chen & Chen, 2005; Lersten & Curtis, 1991; Nagai et al., 2013). The structure of taro hydathodes has been illustrated in previous studies by diagrammatic drawings and light micrographs (Flood, 1919; Müller, 1919). However, the hydathode structure of taro still has room for consideration, and there have been no reports on the hydathode morphology of eddo as far as we know.

Salinity restricts taro cultivation in some areas of Asia and the Pacific, where taro is a staple food (Hill et al., 1998). Plants have different tolerances and response mechanisms to saline conditions. Salinity causes osmotic stress and ion toxicity in plants (Munns & Tester, 2008). One toxic effect of sodium is that excess concentrations inhibit enzyme activity. Mechanisms to reduce salt stress in plants include salt exclusion in the root, sequestration into leaf cell vacuoles, ion retranslocation (Lessani & Marschner, 1978; Lohaus et al., 2000), ion leaching from the leaf apoplast by precipitation (Lohaus et al., 2000; Tukey, 1970), and sodium excretion through hydathodes (Klepper & Kaufmann, 1966; Shabala

et al., 2010; Shapira et al., 2009). Plants generally maintain low sodium levels in the cytoplasm and/or high potassium ion (K$^+$):sodium ion (Na$^+$) ratios that are considered to alleviate salt injury. Sodium restricts potassium influx into the plant cell under saline conditions (Kronzucker et al., 2006), thus affecting the K$^+$:Na$^+$ ratio.

Hydathode exudes guttation fluid, which contains many organic and inorganic substances (Goatley & Lewis, 1966; Islam & Kawasaki, 2015; Mizuno et al., 2002; Shapira et al., 2013; Singh & Singh, 2013). Sodium exudation through guttation flow has been shown to increase considerably in some plants under saline conditions (Maathuis et al., 2014). However, guttation was shown to cease finally under increasing salinity in rice seedlings (Doi & Kasaku, 1953) and barley seedlings (Shabala et al., 2010). At present, it is unknown how much sodium is excreted in guttation fluid through hydathodes in eddo.

In barley, hydathodes were shown to excrete chlorine ions under high humidity through guttation flow (Nagai et al., 2013). Islam and Kawasaki (2015) suggested that guttation eliminates excess calcium, thereby maintaining calcium ion homeostasis in eddo. At present, the role of guttation in balancing sodium concentrations in eddo is unclear. To understand the mechanisms of salinity tolerance and solute movement in eddo, it is important to analyze the morphology of hydathodes and evaluate their role in sodium excretion via guttation fluid. Thus, in the present study, the detailed morphological structure of eddo hydathodes was observed using not only light microscopes but also an electron microscope. We also subjected eddo plants to varying degrees of NaCl stress to evaluate the role of guttation in distributing and balancing sodium.

1. Materials and methods

1.1. Experimental site and plant materials

The experiment was conducted at Hirosaki University (40°59′N, 140°47′E; 53 m above sea level), Hirosaki, Japan. Seed corms of eddo [*C. esculenta* (L.) Schott var. *antiquorum* Hubbard & Rehder cv. Aichiwase] were planted in plastic pots filled with vermiculite in April 2015 and grown under ambient temperature, light, and humidity conditions in a greenhouse for two months, with watering as required. Then, the plants were transferred to a hydroponic solution (Kawasaki et al., 2008) to recover from transplant injury in a growth chamber (MLR-351H; Sanyo, Osaka, Japan) for 7 d with continuous aeration under the following growth conditions: 25/20 °C day/night temperatures, 16 h light/8 h dark photoperiod with light at approximately 150 μmol m^{-2} s^{-1}, and 60% relative humidity. The basic hydroponic solution contained .10 mM sodium. At end of

this recovery period, the plants were at the three- to four-leaf stage and had reached 22–30 cm in height. The average length of leaves was 8.65 cm (sinus to tip). These plants were used for sodium chloride treatments as described below. Seed corms of the same variety were also sown in the field of Hirosaki University campus in May 2015 with recommended fertilizers and necessary intercultural operations. These field-grown plants were used to study the morphology of eddo hydathodes.

1.2. Observations of external morphology of hydathodes

Whole leaves and the leaf tips of the young plants grown in plastic pots, as described above, were photographed using a digital camera and a stereomicroscope (SZX16; Olympus, Tokyo, Japan). The average length of these leaves was 8.63 cm (sinus to tip). For scanning electron microscopy, eddo plants were also grown in field conditions and mature leaves (25–30 cm from sinus to tip) were collected. Segments of the tip, margin, and center parts of leaf blades were frozen rapidly in liquid nitrogen, and then freeze-dried at 25–35 Pa at −44 °C for 72 h using an FDU-1200 vacuum freeze dryer (Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The dried leaf blade samples were mounted on stubs with conductive carbon tape, coated with platinum using an auto fine coater (JFC-1600; JEOL, Tokyo, Japan), and observed under a scanning electron microscope (JSM-7000F; JEOL) at an accelerating voltage of 5 kV. These analyses allowed us to determine the distribution of hydathodes and to view their external structure. One leaf blade per plant in the 0 (control) and 12 mM NaCl treatments and five plants per treatment were analyzed. The distances to the nearest and furthest hydathode pore from the leaf tip edge and the pore diameter of hydathodes were determined using Image J software.

1.3. Observations of internal morphology of hydathodes

Segments of the tip, margin, and center parts of leaf blades from field-grown eddo plants were immersed in .05 M sodium phosphate buffer (pH 7.0) containing 2% (v/v) paraformaldehyde and 1% (v/v) glutaraldehyde at 20 °C for 4 h. Then, samples were washed in .05 M sodium phosphate buffer. The materials were post-fixed in 1% (w/v) osmium tetroxide in .1 M sodium phosphate buffer at 20 °C for 2 h, rinsed in distilled water, and then immersed in 15, 25, and then 50% (v/v) dimethyl sulfoxide, each for 30 min. Then, samples were frozen quickly in droplets of 50% (v/v) dimethyl sulfoxide with liquid nitrogen and cracked cross-ways or longitudinally with a razor blade. After melting, the samples were rinsed in distilled water,

and then immersed in .1 M sodium phosphate buffer containing 1% (w/v) osmium tetroxide at 4 °C for 10 h. Then, they were washed in .1 M sodium phosphate buffer and distilled water. The samples were dehydrated in a graded ethanol series, followed by 100% isoamyl acetate, and then dried using a critical point dryer (JCPD-5, JEOL). As described above, the cracked surface of samples was observed under a scanning electron microscope at an accelerating voltage of 5 kV. One leaf blade per plant and five plants per treatment were analyzed.

Similarly, 6–8 mm^2 samples were taken from the tip, margin, and center parts of leaf blades from field-grown eddo plants for bright-field optical microscopy. The samples were immersed in .05 M sodium phosphate buffer (pH 7.0) containing 2% (v/v) paraformaldehyde and 1% (v/v) glutaraldehyde at 4 °C for 10 h. Then, samples were washed with .05 M sodium phosphate, before being fixed in 1% (w/v) osmium tetroxide in .1 M sodium phosphate buffer at 20 °C for 2 h, and then washed in .1 M sodium phosphate buffer. Samples were dehydrated in a graded ethanol series, followed by propylene oxide, and then embedded in Spurr's resin at 70 °C for 24 h. Then, samples were cut with glass knives into 1-μm-thick sections using an ultramicrotome (Leica EM UC7, Nussloch, Germany) and stained with .1% (w/v) toluidine blue-O. The sections were observed under a bright-field optical microscope (BX51; Olympus) and photographed. One leaf blade per plant and five plants per treatment were investigated. The hydathode cavity area of the longitudinal and cross sections was measured using Image J software.

1.4. Sodium chloride treatments

After the recovery period as described above, the plants were treated for 7 d in hydroponic solutions containing 0 (control), 1, 4, 8, and 12 mM NaCl. All of these concentrations represented low levels of salinity because there were no visible symptoms of salt damage on the eddo plants in this experiment. In addition, the volume of guttation fluid decreased with increasing NaCl levels in the hydroponic solution and very little guttation fluid was exuded from plants in the 12 mM NaCl treatment. Thus, we used the five concentrations of NaCl to evaluate the function of guttation at low salinity levels. There were six plants per treatment, and the growth conditions in this treatment period were the same as those described above, except that the relative humidity was increased to 85% to create suitable conditions for guttation. Hydroponic nutrient solutions were changed every evening to prevent a lack of oxygen and to maintain consistency among treatments. The treatment solutions were maintained at about pH 5.2. Guttation fluid was collected from the hydathodes at the leaf tip into a small poly packet and collected everyday in the morning during the treatment period. Plants were also sampled after the NaCl treatments and used for analyses.

1.5. Measurement of sodium and potassium contents in leaf blades, petioles, corms, and roots

After the NaCl treatments, the roots were washed three times with distilled water. The plants were then separated into leaf blades, petioles, corms, and roots. These samples were dried at 60 °C for 3 d in an oven (DV 340S, Yamato Scientific Co., Tokyo, Japan) and then ground. A portion of the powder (.1 g) was digested with 1% (v/v) hydrochloric acid containing 1000 ppm lanthanum chloride with shaking for 4 d. The digested solution was filtered through a cellulose membrane syringe filter (.45 μm pore size). The sodium and potassium concentrations of the filtered solution of leaf blades, petioles, corms, and roots were measured using a flame atomic absorption spectrophotometer (Z-2000; Hitachi, Tokyo, Japan). All leaf blades, petioles, corms, and roots of each plant were used, and six plants per treatment were analyzed. Whole-plant sodium and potassium contents were calculated based on the total dry weights of leaf blades, petioles, corms, and roots of eddo plants.

1.6. Measurement of sodium and potassium contents of guttation fluid

The volume of guttation fluid per plant treated by NaCl was measured with a graduated measuring cylinder. Guttation fluid containing 1000 ppm lanthanum chloride was filtered through a cellulose membrane syringe filter and analyzed to determine sodium and potassium contents as described above. Guttation fluid from all leaves of six plants per treatment was analyzed.

The ratios of total sodium amounts in guttation fluid eliminated through hydathodes during the 7-d treatment period to sodium contents of leaf blades in a plant, leaves (leaf blades and petioles) in a plant, and the whole plant after the treatments were calculated. The ratios of potassium to sodium contents were obtained from these contents in guttation fluid, leaf blades, petioles, corms, roots, and the whole plant.

1.7. Statistical analysis

Data for guttation volumes, sodium concentrations, ratios of sodium contents of guttation fluid to those in leaf blades, leaves, and whole plants, and ratios of potassium to sodium contents in guttation fluid and other plant parts were subjected to analysis of variance, followed by Tukey's test.

2. Results

2.1. External structure of eddo hydathode

The eddo leaf blade is heart-shaped, with an entire margin and reticulate venation (Figure 1(a)). The tip of the leaf blade looks like a spur or a hull-shaped depression (Figures 1(b) and (c) and 2(a)). In our electron microscope analyses, we observed that about five hydathode pores were located at a distance of about 1–2 mm from the tip edge on the adaxial surface of a mature leaf (Table 1, Figure 2(a)). Hydathode pores were ovoid to almost circular with a diameter ranging from 33 to 95 μm (Table 1, Figure 2(a)–(c)). Externally, hydathode pores appeared to be permanently open and were surrounded by a ring structure (Figures 1(d) and 2(b) and (c)). Electron micrographs revealed two borders on the surface of the

ring structure (Figure 2(b) and (c)). In our study, we did not observe hydathode pores along the margin or on the adaxial or abaxial surfaces of the eddo leaf blades (Figure 2(e) and (f)). Many stomata were present on the adaxial or abaxial surfaces of the leaf blade but not at the leaf tip (Figure 2(a) and (d)–(f)).
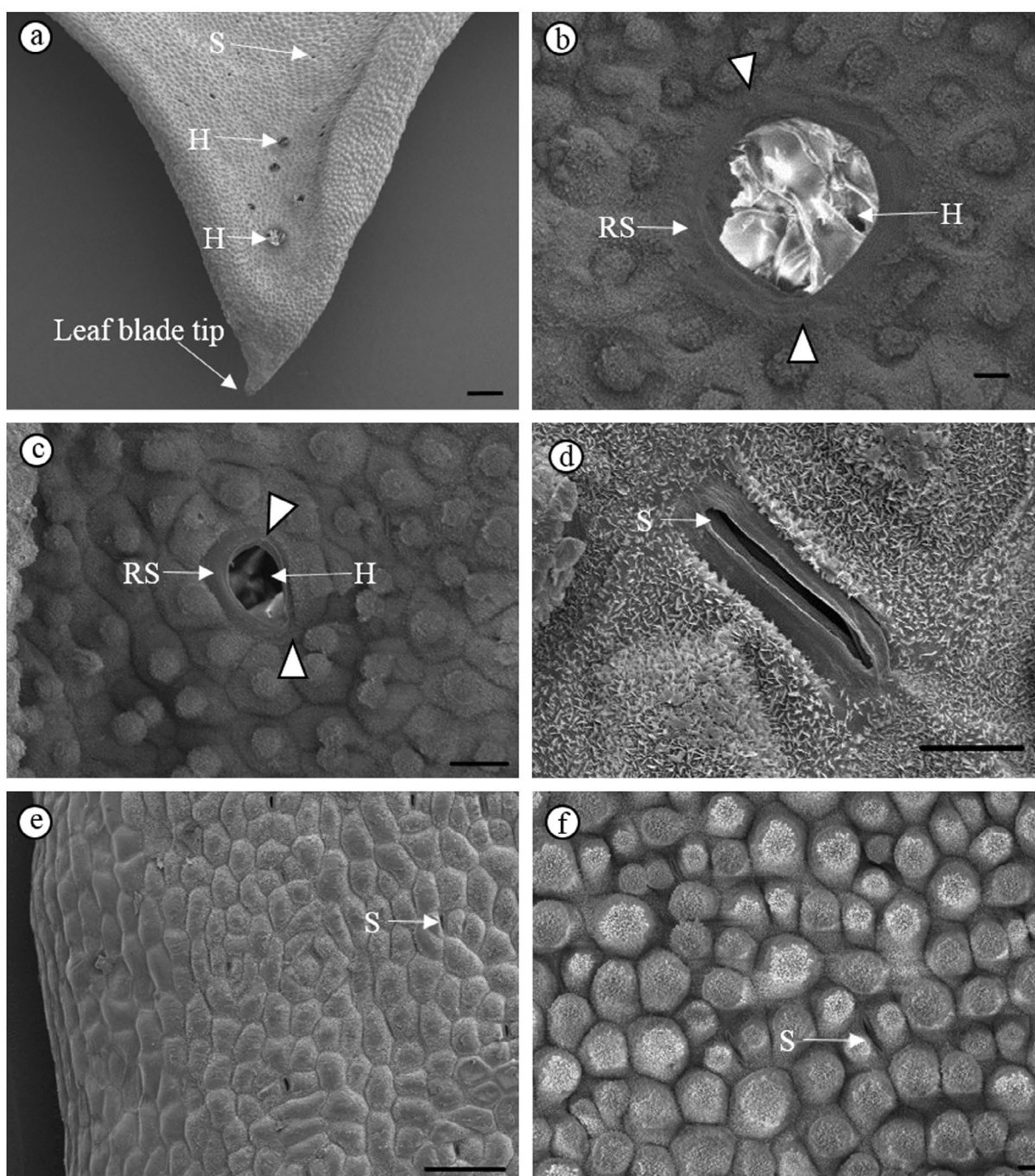
2.2. Internal structure of hydathode

Each hydathode pore was surrounded by cells. At the mature stage, these cells sometimes shrunk (Figure 3(a)–(c)). There was a large cavity underneath the hydathode pore in eddo (Figures 3(a)–(c) and 4(a) and (b)). As shown in Table 1, the hydathode cavity area was estimated to be .47 and .39 mm^2, based on measurements from longitudinal and cross sections, respectively. Some parenchyma
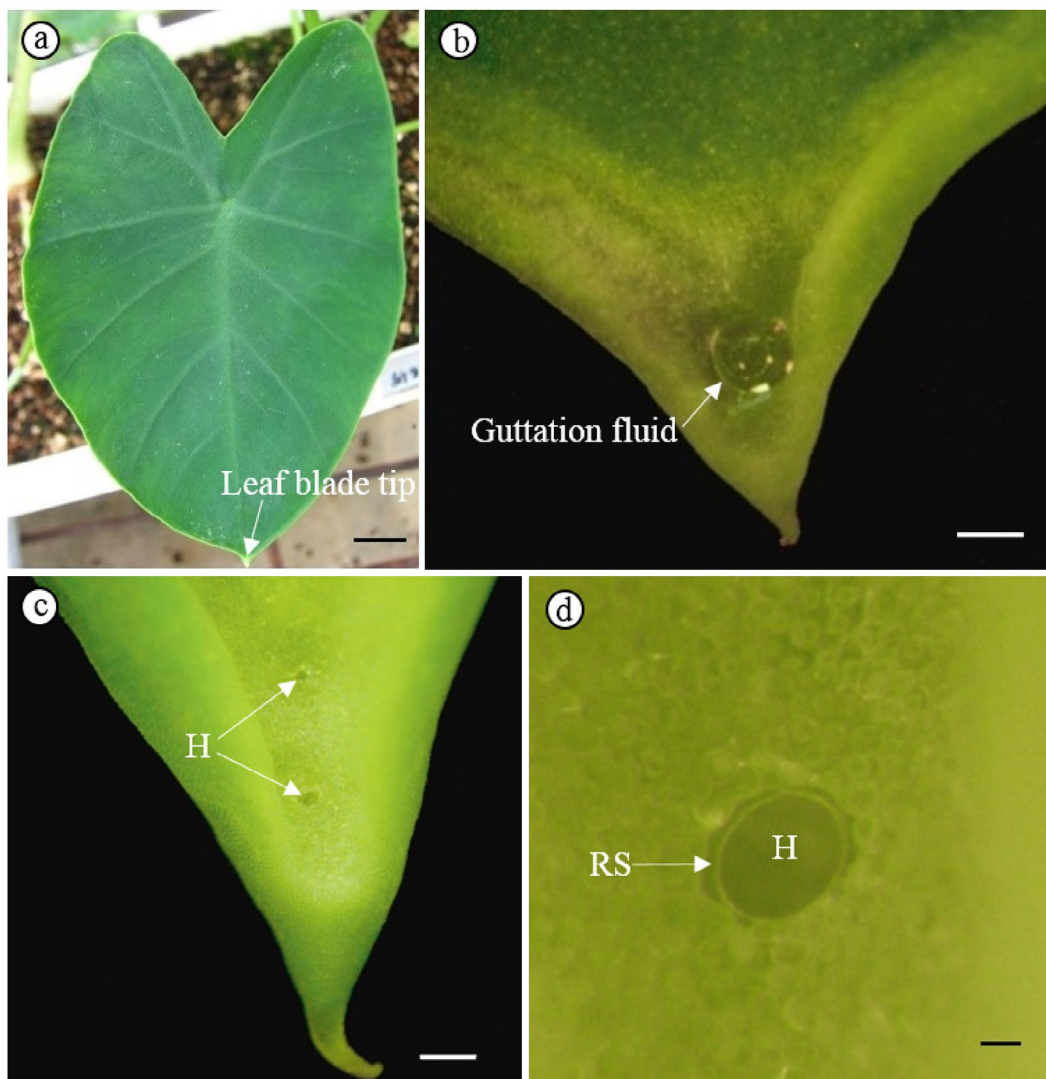
Figure 1. Photograph of eddo leaf blade and stereo-micrographs of leaf blade tip.
Notes. (a) Leaf blade, bar = 1 cm. (b) Leaf blade tip with a drop of guttation fluid, bar = 500 μm. (c) Leaf blade tip of adaxial surface with hydathodes, bar = 250 μm. (d) Enlarged view of hydathode pore, bar = 10 μm. H, Hydathode pore; RS, Ring structure.

Figure 2. Scanning electron micrographs of external structures of hydathode and leaf blade.
Notes. (a) Hydathode pores at the tip of adaxial surface, bar = 100 μm. (b) Large hydathode pore with ring structure, bar = 10 μm. (c) Small hydathode pore with ring structure, bar = 10 μm. (d) Stoma, bar = 10 μm. (e) Abaxial surface of leaf blade margin, bar = 100 μm. (f) Adaxial surface of center part of leaf blade, bar = 100 μm. Arrowhead indicates border of ring structure. H, Hydathode pore; RS, Ring structure; S, Stomata.

Table 1. Number, position from leaf tip, pore diameter, and cavity area of hydathodes in mature leaf blades of eddo.

	Distance (mm) from tip to		Pore diameter (μm)		Cavity area (mm^2)	
Hydathode number	Nearest hydathode	Furthest hydathode	Smallest hydathode	Largest hydathode	Longitudinal section	Cross section
5 ± 3	1.08 ± .24	1.97 ± .44	33 ± 15	95 ± 28	.47 ± .05	.39 ± .06

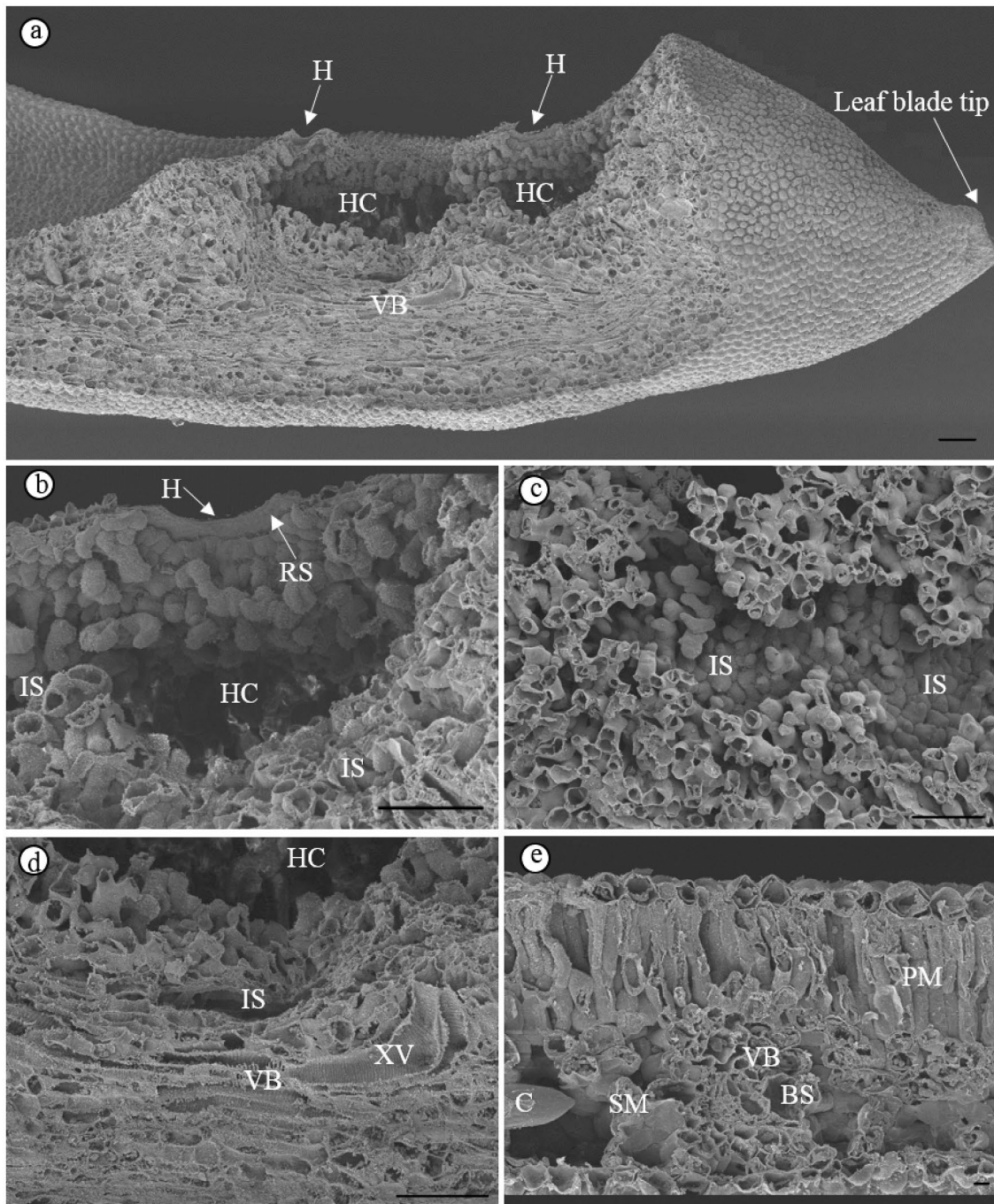*Note.* Values are mean ± SD (*n* = 7).
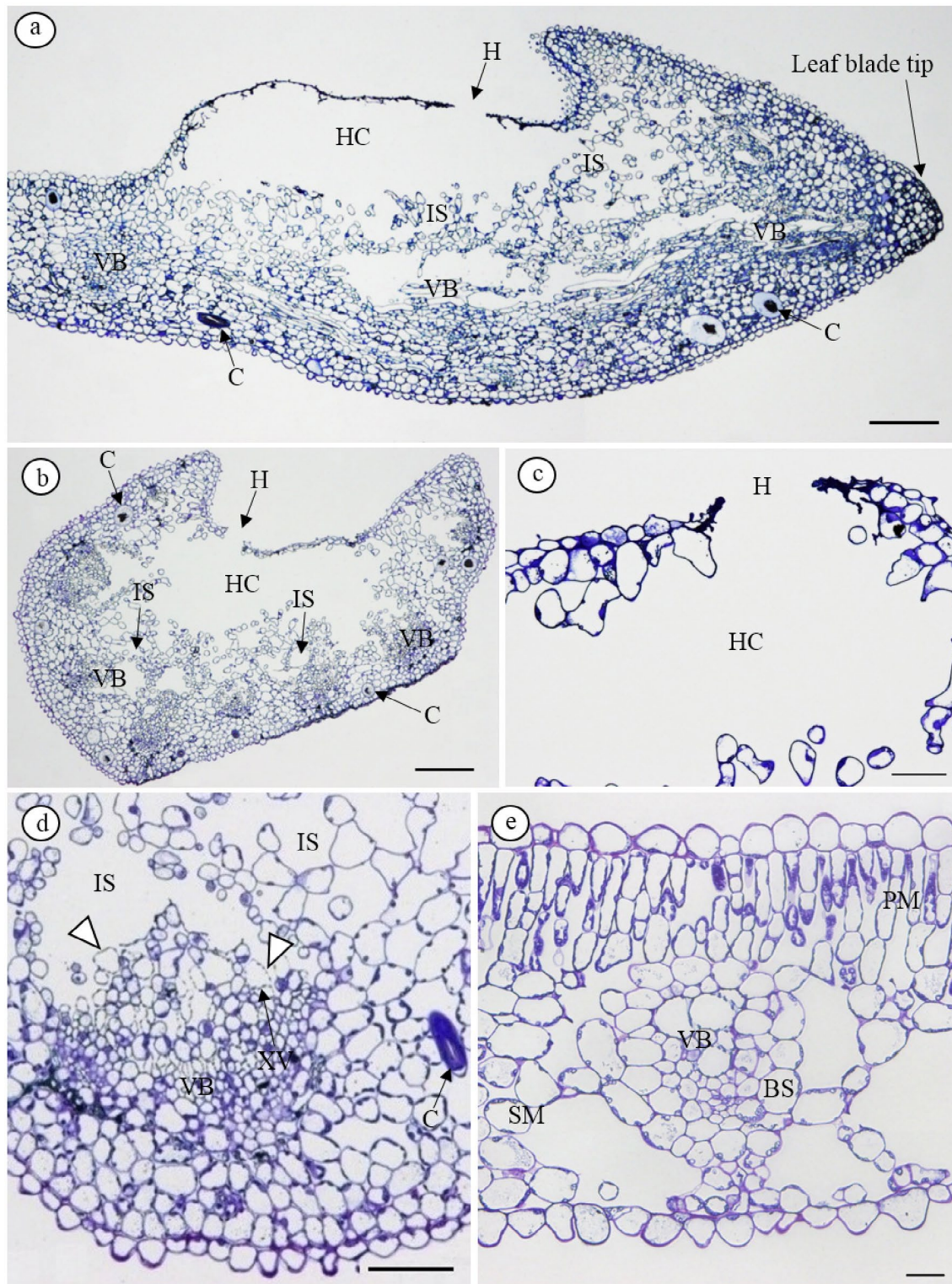
Figure 3. Light micrographs of internal structure of hydathode and leaf blade.
Notes. (a) Longitudinal section of leaf blade tip with hydathode, bar = 200 µm. (b) Cross section of leaf tip with hydathode, bar = 200 µm. (c) Hydathode pore and cavity in longitudinal section, bar = 50 µm. (d) Cross section of vascular bundle near hydathode area, bar = 100 µm. (e) Cross section of center part of leaf blade, bar = 50 µm. Arrowheads indicate connection between vascular vessels and intercellular spaces. BS, Bundle sheath; C, Crystal cell; H, Hydathode pore; HC, Hydathode cavity; IS, Intercellular spaces in loosely arranged parenchyma cells; PM, Palisade mesophyll; SM, Spongy mesophyll; VB, Vascular bundle; XV, Xylem vessel.

cells were loosely arranged, creating intercellular spaces between the cavity and vascular bundles (Figures 3(a) and (b) and 4(a) and (c)). The intercellular spaces connected the vessel elements to the cavity (Figures 3(a), (b) and (d), 4(c) and (d)). The hydathode area did not have distinct palisade and spongy mesophyll layers (Figures 3(a) and

Figure 4. Scanning electron micrographs of internal structure of hydathode and leaf blade.

Notes. (a) Longitudinally cracked part of leaf blade tip with hydathodes, bar = 100 μm. (b) Hydathode pore with ring structure and hydathode cavity, bar = 10 μm. (c) Loosely arranged parenchyma cells with intercellular spaces between hydathode cavity and vascular bundles, bar = 100 μm. (d) Vascular bundles connected to intercellular spaces of parenchyma cells, bar = 100 μm. (e) Cross-cracked part of leaf blade center area, bar = 100 μm. BS, Bundle sheath; C, Crystal cell; H, Hydathode pore; HC, Hydathode cavity; IS, Intercellular spaces in loosely arranged parenchyma cells; PM, Palisade mesophyll; RS, Ring structure; SM, Spongy mesophyll; VB, Vascular bundle; XV, Xylem vessel.

(b) and 4(a)), like those observed in the center part of the leaf blade (Figures 3(e) and 4(e)). The vascular bundles in the center part of the leaf blade had a bundle sheath (Figures 3(e) and 4(e)), but those near the hydathode had no bundle sheath (Figures 3(d) and 4(d)). There were many crystal cells around the hydathode cavity and vascular bundles (Figure 3(a), (b) and (d)).

2.3. Sodium contents and distribution in leaf blades, petioles, corms, and roots

As the NaCl concentration in the hydroponic solution increased, the sodium contents increased significantly in the leaf blades, petioles, and roots (Figure 5). The sodium contents in the leaf blade and petiole were .23
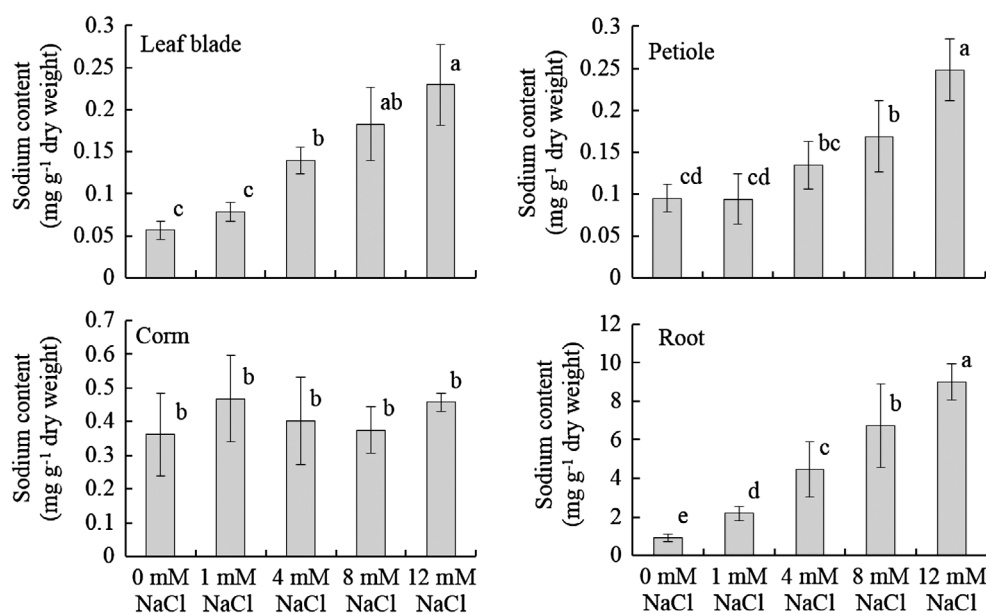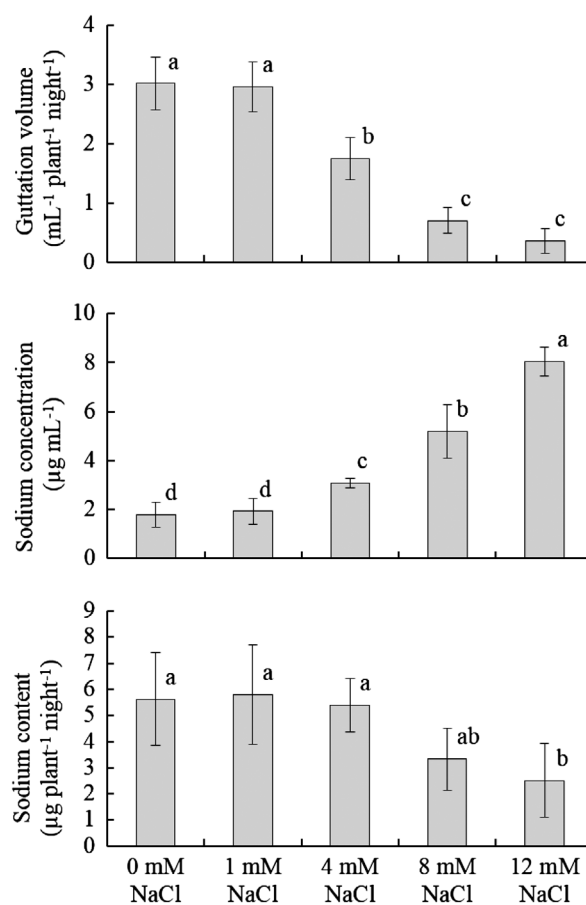
Figure 5. Sodium contents in eddo leaf blades, petioles, corms, and roots.
Notes. Vertical bars represent mean ± SD (*n* = 6). Mean values with same letters are not significantly different at 5% level (Tukey's test).

and .25 mg g^{-1} dry weight (DW), respectively, in the 12 mM NaCl treatment, compared with .06 and .09 mg g^{-1} DW, respectively, in the control. The sodium content in the roots was 9.01 mg g^{-1} DW in the plants treated with 12 mM NaCl, whereas the roots contained only .93 mg g^{-1} DW in the control. Compared with the control plants, those in the 12 mM NaCl treatment had 3.83, 2.78 and 9.69 times higher sodium contents in the leaf blades, petioles, and roots, respectively. However, there was no significantly difference in the sodium content of corms among NaCl treatments (Figure 5). The sodium content in corms was .46 mg g^{-1} DW in the 12 mM NaCl treatment and .36 mg g^{-1} DW in the control.

2.4. Guttation fluid volume, sodium concentration in guttation fluid, and contribution of guttation to sodium elimination

In our experiment, eddo plants in the 0 mM NaCl (control) and 1 mM NaCl treatment exuded the largest volume of guttation fluid (3.01 and 2.96 mL plant^{-1} night^{-1}, respectively, Figure 6). However, the volume of guttation fluid decreased significantly as the NaCl concentration in the hydroponic solution increased from 1 to 12 mM. The sodium concentrations in guttation fluid were the lowest in the control and 1 mM NaCl treatments (1.78 and 1.93 µg mL^{-1}, respectively), and increased gradually as the NaCl concentration in the hydroponic solution increased, reaching a maximum in the 12 mM NaCl treatment (8.04 µg mL^{-1}) (Figure 6). The amount of sodium eliminated via guttation decreased significantly from 5.38 µg plant^{-1} night^{-1} in the 4 mM NaCl treatment to 2.51 µg plant^{-1} night^{-1} in the 12 mM NaCl treatment (Figure 6).

Figure 6. Guttation volume and contribution of guttation to sodium elimination.
Notes. Guttation volume, sodium concentration in guttation fluid and sodium content in guttation fluid excreted nightly through hydathodes of leaves of eddo plants treated with different levels of NaCl in hydroponic solution. Vertical bars represent mean ± SD (*n* = 6). Mean values with same letters are not significantly different at 5% (Tukey's test).

2.5. Ratios of sodium content in guttation fluid to that in leaf blades, leaves, and whole plants

As shown in Table 2, the ratios of sodium content in the guttation fluid to sodium content in leaf blades, leaves (leaf blade and petiole), and whole plants were 81.50, 30.31, and 3.51%, respectively, in the control plants at the end of the 7-d experimental period. As the concentration of NaCl in the hydroponic solution increased, the ratios of sodium content in guttation fluid to that in leaf blades, leaves, and whole plants decreased (Table 2). In the plants in the 12 mM NaCl treatment, the ratios of sodium content in the guttation fluid to sodium contents in leaf blades, leaves, and whole plants were 7.66, 3.79, and .16%, respectively. In all treatments, the ratio of sodium content of guttation fluid to that in the leaf blade was much higher than the ratio of sodium content in guttation fluid to sodium content in whole plants.

2.6. Ratio of potassium to sodium contents in plant tissues and guttation fluid

Table 3 shows the ratios of potassium to sodium contents in leaf blades, leaves, corms, roots, whole plants, and guttation fluid of eddo grown in hydroponic solution with NaCl at various concentrations for 7 d. The ratio in the guttation fluid and all plant parts except the corms decreased with increasing NaCl levels in the hydroponic solution. The NaCl concentration did not significantly affect the ratio in corms. In leaf blades, leaves, and roots, the ratios of potassium to sodium contents were significantly lower in the 4, 8, and 12 mM NaCl than in the 0 and 1 mM NaCl treatments. On the whole-plant level, the ratio of potassium to sodium content was significantly lower in the 12 mM NaCl than in the 0 and 1 mM NaCl treatments, but did not differ significantly among the 1, 4, and 8 mM NaCl treatments. In the guttation fluid, the ratio of potassium to sodium contents in the control was significantly higher than that in the 4, 8, and 12 mM NaCl treatments (Table 3). Among the plant parts, the roots showed the lowest potassium to sodium ratio in each NaCl treatment.

3. Discussion

Hydathodes are present at the tip, on the adaxial and/or abaxial surface, and along the margin of leaves in a wide range of plant species (Chen & Chen, 2005; Lersten & Curtis, 1991; Maeda & Maeda, 1987; Taiz & Zeiger, 2006). Hydathode pores are located at the apex of the adaxial leaf surface in taro (Flood, 1919). In eddo, the hydathode pores are also arranged along the leaf tip on the adaxial surface (Figures 1(c) and 2(a)), and not along the margin or on the adaxial or abaxial surfaces of leaf blade (Figure 2(e) and (f)). Chen and Chen (2005) revealed that a large mature leaf of *Ficus formosana* (L.) has about 1000 hydathode pores. A taro leaf has one to five hydathode pores with a diameter of 100 μm (Flood, 1919). Our study showed that, in eddo, about five permanently open hydathode pores with a diameter of 33–95 μm are located 1–2 mm from the tip edge of the mature leaf blade (Table 1).

In this study, a ring structure around the hydathode pore and two borders on the surface of the ring were found by scanning electron microscopy (Figure 2(b) and (c)). Observations using a bright-field optical microscope showed that the hydathode pore in eddo is surrounded by cells (Figure 3(a)–(c)). Pillitteri et al. (2008) demonstrated that mature guard cells and hydathodes share similar gene

Table 2. Ratios of sodium content in guttation fluid to that in leaf blades, leaves (leaf blade and petiole), and whole plants.

	Ratios of sodium content (%)		
Treatments	Guttation fluid to leaf blades in a plant	Guttation fluid to leaves in a plant	Guttation fluid to a whole plant
0 mM NaCl	81.50 ± 23.41 a	30.31 ± 12.74 a	3.51 ± .92 a
1 mM NaCl	77.69 ± 18.79 a	33.84 ± 10.95 a	1.73 ± .55 b
4 mM NaCl	35.44 ± 6.46 b	17.07 ± 3.24 b	1.11 ± .38 bc
8 mM NaCl	19.68 ± 8.97 bc	9.07 ± 1.73 bc	.39 ± .22 cd
12 mM NaCl	7.66 ± 3.44 c	3.79 ± 1.59 c	.16 ± .08 d

Notes. Values are mean ± SD ($n = 6$). Mean values with same letters within a column are not significantly different at 5% (Tukey's test).

Table 3. Effect of NaCl at different concentrations on the ratios of potassium to sodium content of eddo plant tissues and guttation fluid.

	Potassium:sodium ratio in					
Treatments	Leaf blade	Leaf	Corm	Root	Whole plant	Guttation fluid
0 mM NaCl	594.45 ± 134.22 a	651.13 ± 99.04 a	65.29 ± 20.48 a	52.56 ± 8.64 a	138.30 ± 17.05 a	.99 ± .40 a
1 mM NaCl	412.05 ± 83.03 b	601.29 ± 80.38 a	50.42 ± 26.10 a	22.96 ± 5.90 b	62.07 ± 23.21 b	.76 ± .33 ab
4 mM NaCl	229.35 ± 80.62 c	365.72 ± 121.43 b	51.13 ± 31.10 a	12.69 ± 6.67 c	48.95 ± 24.68 bc	.41 ± .15 bc
8 mM NaCl	208.57 ± 103.51 c	316.30 ± 73.27 b	48.48 ± 10.77 a	8.88 ± 4.20 c	32.91 ± 17.67 bc	.33 ± .13 c
12 mM NaCl	186.06 ± 77.20 c	238.59 ± 47.31 b	44.34 ± 6.76 a	5.22 ± .82 c	24.38 ± 3.75 c	.18 ± .10 c

Notes. Values are mean ± SD ($n = 6$). Mean values with same letters within a column are not significantly different at 5% (Tukey's test).

expression patterns, and that hydathode pores and stomata share a similar developmental pathway. Müller (1919) showed a sketch of the guard cells-like structure around hydathode pore in *Ariopsis* leaves. Our results showed that two large cells, which may be modified guard cells, surround the hydathode pore in leaf blades of eddo.

In mature strawberry leaves, there is a cavity underneath the hydathode pore and above the epithem cells (Takeda et al., 1991). We estimated the area of this cavity in strawberry to be about .06–.07 mm^2, based on analyses of cross sections in the thesis of Takeda et al. (1991). The area of the hydathode cavity in eddo is comparatively very large (.39–.47 mm^2) (Table 1). The hydathodes of *F. formosana* have some epithem cells and their associated vascular bundles have a bundle sheath (Chen & Chen, 2005). Flood (1919) and Müller (1919) reported that the hydathodes of taro lack epithem cells and a bundle sheath. In this study, we did not observe structures resembling epithem cells or a bundle sheath around the vascular bundle in the tip part of the leaf blade (Figures 3(d)–(e) and 4(d)–(e)). We observed loosely organized parenchyma with intercellular spaces between the hydathode cavity and vascular bundles in the tip of the eddo leaf blade (Figures 3(d) and 4(c)). Maeda and Maeda (1988) revealed that the hydathode pore on rice leaf blades is directly connected to vessel elements via intercellular spaces. We also observed many crystal cells near the hydathodes (Figure 3(a), (b) and (d)). The light and electron microscope analyses conducted in this study have provided a much greater understanding of hydathode morphology in eddo.

In eddo, the hydathodes on the leaf blades exude guttation fluid as part of their normal growth. Shapira et al. (2009) reported that banana leaves exuded guttation fluid under mild salinity (2 mM NaCl), but ceased exuding guttation fluid as the degree of salinity increased. Higher NaCl treatments were also shown to suppress guttation in barley (Shabala et al., 2010) and rice seedlings (Doi & Kasaku, 1953). In our study, the volume of guttation fluid exuded by control plants was 3.01 mL^{-1} plant^{-1} night^{-1}, but significantly lower in plants under increasing levels of NaCl in hydroponic solution (Figure 6). The eddo plants in the 12 mM NaCl treatment exuded .36 mL^{-1} plant^{-1} night^{-1}. Shabala et al. (2010) showed that the sodium content in guttation sap increased 10–15 times in barley seedlings under salinity stress. In this study, as the NaCl concentrations in the hydroponic solution increased, the sodium concentration in guttation fluid increased, but the volume of guttation fluid decreased (Figure 6). Consequently, the total amount of sodium eliminated through guttation fluid decreased. There were no significant differences in sodium contents in guttation fluid among the 0, 1, 4, and 8 mM NaCl treatments, but the amount of sodium excreted was higher in the 4 mM NaCl treatment (5.38 µg plant^{-1} night^{-1})

than in the 12 mM NaCl treatment (2.51 µg plant^{-1} night^{-1}) (Figure 6). The sodium content in the leaf increased with increasing NaCl concentrations in the hydroponic solution (Figure 5). Maathuis et al. (2014) noted that 2–4% of the total sodium content in tomato plants was excreted via the guttation fluid, and that guttation could not be assumed to relieve salinity stress. In the present study, the ratios of the sodium content in guttation fluid to that in leaf blades decreased from 81.50 to 7.66% with increasing NaCl levels in the hydroponic solution, but this represented only 3.51–.16% of the sodium content of the whole eddo plant (Table 2). These data indicated that while guttation eliminated some sodium, it could not maintain sodium homeostasis in eddo plants under saline conditions.

The cytosolic K$^+$:Na$^+$ ratio is a key determinant of plants' ability to survive under saline conditions (Colmer et al., 2006; Gorham et al., 1991; Maathuis & Amtmann, 1999; Shabala & Cuin, 2007). Salinity was shown to enhance the sodium content and decrease the K$^+$:Na$^+$ ratio in *Vicia faba* (Gadallah, 1999). Tunçtürk et al. (2011) found that the K$^+$:Na$^+$ ratio decreased in *Brassica napus* under saline conditions. In barley, epithem cells around the hydathode were shown to store or re-export phosphate and potassium ions to other plant parts, while chlorine ions, which are toxic in excess concentrations, were excreted in guttation fluid through the hydathodes (Nagai et al., 2013). Our experimental data showed that the ratio of potassium to sodium contents decreased in most plant parts of eddo, except for the corms, with increasing NaCl levels in hydroponic solution (Table 3). The ratio in the guttation fluid of eddo also decreased with increasing salinity. Therefore, guttation did not play a role in adjusting the ratio of potassium to sodium contents in eddo plants under saline conditions.

There are many salinity tolerance and response mechanisms in plants, such as ion homeostasis and compartmentalization, ion transport and uptake, and sodium excretion through hydathodes (Gupta & Huang, 2014; Klepper & Kaufmann, 1966; Shapira et al., 2009). In this study, our results show that hydathodes do not play a role in sodium homeostasis in eddo. In addition, sodium contents increased in roots and remained stable in corms with increasing sodium concentrations in the hydroponic solution, but slowly increased in leaf blades and petioles (Figure 5). Therefore, it is suggested that the lower levels of sodium in leaves results from restriction of sodium transport from roots to leaves. This information on the movement of sodium from the entry point (roots) to the outlet (hydathode) under saline conditions is valuable for understanding the mechanism of salt response and tolerance in eddo. Our results related with morphology and physiology will be useful for thinking the improvement of salinity tolerance in eddo.

Acknowledgment

Disclosure statement

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