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PETIOLE BENDING IN DETACHED LEAVES OF *BRYOPHYLLUM CALYCINUM*: RELEVANCE TO POLAR AUXIN TRANSPORT IN PETIOLES

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This manuscript is dedicated to the memory of the late Doc. dr hab. Alicja Saniewska.

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Petiole bending in detached leaves of *Bryophyllum calycinum* was intensively investigated in relation to polar auxin transport in petioles. When detached leaves were placed leaf blade face down, clear petiole bending was observed. On the other hand, no petiole bending was found when detached leaves were placed leaf blade face up. Indole-3-acetic acid (IAA) exogenously applied to petioles was significantly effective to induce and/or stimulate petiole bending when detached leaves were placed leaf blade face down. To clarify the mechanisms of petiole bending in detached leaves of *B. calycinum* when they were placed leaf blade face down, the effects of application of IAA, ethephon which is an ethylene releasing compound, inhibitors of polar auxin transport such as 2,3,5-tiiodobenzoic acid (TIBA), *N*-1-naphthylphthalamic acid (NPA) and 9-hydroxyfluorene-9-carboxylic acid (HFCA) and methyl jasmonate (JA-Me) were thoroughly investigated. Ethephon was not effective to enhance petiole bending, suggesting that ethylene derived from exogenously applied IAA does not play an important role in petiole bending in detachd leaves of *B. calycinum*. This suggestion was strongly supported by the fact that ethephon exogenously applied to petioles in intact plant of *B. calycinum* had no effect on inducing epinasty and/or hyponasty either (Ueda et al., 2018). Potent inhibitors of polar auxin transport, TIBA and HFCA, and JA-Me were extremely effective to inhibit petiole bending but NPA was not. Almost no petiole bending was observed in excised petiole segments without the leaf blade. Applicaton of IAA to the cut surface of petioles in the leaf blade side strongly promoted petiole bending. Polar auxin transport in excised petioles of *B. calycinum* was intensively investigated using radiolabeled IAA ([1-^{14}C] IAA). Clear polar auxin transport was observed in excised petiole segments, indicating that auxin allows movement in one direction: from the leaf blade side to the stem side in petioles. When detached leaves were placed only leaf blade face down, transported ^{14}C-IAA was reduced in the lower side of the excised petioles. These results strongly suggest that transport and/or lateral movement of endogenous auxin biosynthesized or produced in the leaf blade are necessary to induce petiole bending in detached leaves of *B. calycinum*. Mechanisms of petiole bending in detached leaves of *B. calycinum* are also discussed in relation to polar auxin transport and lateral movement of auxin.

Keywords: *Bryophyllum calycinum*, detached leaves, ethylene, indole-3-acetic acid, petiole bending, polar auxin transport

INTRODUCTION

Hyponastic and epinastic growth in plants is, respectively, induced by upward and downward petiole movement in response to various external stimuli such as light, temperature, gaseous constituents, moisture status and so on. It is caused by asymmetrical growth rates between adaxial and abaxial sides of the petiole. Epinastic growth is considered to be induced by the fact that the growth of the upper side in petioles is greater than that of the lower side, resulting in

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leaf drop. It has been well known that auxin and ethylene are key compounds to induce nastic, especially epinastic growth in plants (Harvey, 1915; Kazemi and Kefford, 1974; Leather et al., 1972). This finding is supported by the fact that partial reduction was induced by application of silver nitrate which is a potent inhibitor of ethylene action, and also by application of α-aminooxyacetic acid (AOA) or aminoethoxyvinylglycine (AVG) which are inhibitors of endogenous production of ethylene (Amrhein and Schneebeck, 1980; Daniel and Rayle, 1980; Saltveit et al., 1979; Saltveit and Larson, 1983). The epinastic response of poinsettia (*Euphorbia pulcherrima* Willd.) was induced by exogenously applying ethylene as well (Osborne, 1982). In hyponastic growth in *Arabidopsis*, ethylene, the volatile plant hormone, plays an important role in inducing differential petiole growth involving the reorientation of cortical microtubules in petioles (Polko et al., 2012). The adaxial petiole cells responsible for epinastic growth are so-called type III target cells responding to ethylene but the growth depends upon the presence of auxin (Osborne, 1982). A drastic increase in petiole bending of tomato plants (*Lycopersicon esculentum* Mill cv 'Heinz 1350') was found when the plants were treated with 0.5 to 1.0 μM brassinosteroid (BR) accompanied by increasing ethylene production (Schlagnhaufer and Arteca, 1985).

Regulation of epinasty in *B. calycinum* seems to be distinctive and not typical relevance to participation of ethylene compared to that in other plants. Involvement of JA-Me in inducing ethylene production when it was exogenously applied to numerous plant tissues has been reported to strongly inhibit epinasty induced by auxin (IAA) in intact of *B. calycinum*. In addition, ethephon, an ethylene-releasing compound, had almost no effect on induction of epinasty in this plant (Ueda et al., 2018). Application of JA-Me for several hours dampens or stops the circadian petal movement rhythm of *Kalanchoe blossfeldiana* flowers depending on the duration and concentration (Engelmann et al., 1977). To confirm possible involvement of auxin but not ethylene in inducing epinasty in *B. calycinum*, the experimental system using detached leaves of this plant was introduced. Recently, it was found that exogenously applied IAA significantly induced and/or stimulated petiole bending in detached leaves of *B. calycinum*, and ethephon did not but it inhibited it significantly when detached leaves were placed the leaf blade face down (Ueda et al., 2018). To clarify possible involvement of auxin transport in petiole bending of *B. calycinum*, polar auxin transport in excised petiole segments using radiolabeled IAA ([1-^{14}C] IAA) was intensively investigated. Potent inhibitors of polar auxin transport, TIBA, NPA and HFCA, were also applied to such a detached leaves system. Possible mechanisms of petiole bending relevance to auxin transport are also adequately discussed.

MATERIALS AND METHODS

PLANT MATERIALS AND CHEMICAL TREATMENT

Immature and mature detached leaves of *Bryophyllum calycinum* Salisb. were prepared from two- to three-month-old plants which were propagated from epiphyllous buds arising in the marginal notches of the leaves. Detached leaves were placed leaf blade face up (normal position) or face down (inverted position) as described in Ueda et al. (2018). Lanolin pastes containing indole-3-acetic acid (IAA, 0.1 or 0.5%, w/w), ethephon (1%, w/w), methyl jasmonate (JA-Me, 0.1% w/w), 2,3,5-triiodobenzoic acid (TIBA, 0.2%, w/w), *N*-1-naphthylphthalamic acid (NPA, 0.2%, w/w) and 9-hydroxyfluorene-9-carboxylic acid (HFCA, 0.2%, w/w) were applied to almost middle part of the petioles in detached leaves as lanolin pastes. The application method of lanolin paste used in this study was the same as that described in our previous paper (Ueda et al., 2018). Excised petioles with or without partial leaf blade were also used. Detailed explanation of chemical treatment in these explants is indicated in Table 1. The treated plants were kept in trays with several layers of filter papers moistened with distilled water in a greenhouse under natural conditions for several hours from July to August in Skierniewice, Poland. Relative humidity in trays where the treated plants were kept was oversaturated. At the end of experiments, angles in treated plants were measured according to the methods already described (Ueda et al., 2018). Three to five detached leaves were used for each treatment. Detached leaves with or without lanolin were used as control. The data were expressed as average values with standard error of the mean in each treatment.

DETERMINATION OF POLAR AUXIN TRANSPORT IN EXCISED PETIOLE SEGMENTS

Determination of polar auxin transport in excised petiole segments of *B. calycinum* proceeded according to the methods with some modifications. This system has already been confirmed to be very suitable and adequate to determine polar auxin transport (Ueda et al., 1999, 2000, 2013). Twenty mm petiole segments were excised and put into 1.5-mL Eppendorf plastic tubes in down or up orientation of the stem side or the leaf blade side. Some of them were also kept in horizontal position as

TABLE 1. Treatment with JA-Me and IAA of excised petiole segments without (a to d) or with (e to h) partial leaf blade of *B. calycinum*.

Treatment in Fig. 3		Application
a	Control	Only lanolin paste was applied to cut surface and middle of excised petiole segments.
b	JA-Me (0.1%, w/w)	Only JA-Me and lanolin were applied to middle and cut surface of excised petiole segments, respectively.
c	IAA (0.5%, w/w)	Only IAA and lanolin were applied to middle and cut surface of excised petiole segments, respectively.
d	IAA (0.5%, w/w) and JA-Me (0.1%, w/w)	IAA and JA-Me were applied to middle and cut surface of excised petiole segments, respectively.
e	Control	Only lanolin paste was applied to cut surface of leaf blade and middle of excised petiole segments.
f	JA-Me (0.1%, w/w)	Only JA-Me and lanolin were applied to middle of excised petiole segments and cut surface of leaf blade, respectively.
g	IAA (0.5%, w/w)	Only IAA and lanolin were applied to middle of excised petiole segments and cut surface of leaf blade, respectively.
h	IAA (0.5%, w/w) and JA-Me (0.1%, w/w)	IAA and JA-Me were applied to middle and cut surface of excised petiole segments, respectively.

illustrated in Fig. 5. The system consisting of 100 µl of 0.8% agar containing [1-^{14}C] IAA was introduced to determine polar auxin transport. The final concentration of radiolabeled IAA was adjusted to 18.5 MBq/mmol (1µCi/ml). After incubation at ca. 25°C for 18 h in the dark, 2 mm pieces of the opposite side of the segment kept in vertical or horizontal position (as also illustrated in Fig. 5) were cut and directly put into vial containing liquid scintillation cocktails (UniverSol™, MP Biomedicals LLC, Santa Ana, CA, USA). Radioactivities of the small slices were determined by a liquid scintillation counter (2200CA, Hewlett Packard Instruments Co., USA). Radiolabeled IAA with specific activity of 1.85 GBq/mmol (ARC 0160) was purchased from American Radiolabeled Chemicals, Inc., St. Louis, MO, USA.

To clarify auxin distribution in the excised petiole segments, small pieces of the upper or lower sides (5 mm long) at the leaf blade side of the segments were cut into longitudinal halves. Radiolabeled IAA was applied to the cut surface of the upper side or the lower side of the segments. Eppendorf tubes containing excised petiole segments were placed horizontally with leaf blade face up and face down, as illustrated in Fig. 6. After 18 h incubation, the segments were carefully cut into longitudinal halves in the stem sides of petioles. Pieces of petioles (2 mm) were excised from the upper and the lower sides of the segments, as illustrated in Fig. 6. Radioactivities of such two pieces were directly measured by a liquid scintillation counter as described above. The data were expressed as average values of dpm with standard error of the mean in each treatment (n = 5).

RESULTS AND DISCUSSION

PETIOLE BENDING IN DETACHED LEAVES OF *BRYOPHYLLUM CALYCINUM*

When detached leaves were placed not only leaf blade face down but also in vertical position, clear petiole bending was observed; it became convex on the lower, and concave on the upper side of the petioles (Fig. 1). However, no petiole bending was found when the detached leaves were placed leaf blade face up as already shown (Ueda et al., 2018). When the detached leaves were placed leaf blade face up, no petiole bending was found at 21 days of incubation. Since this discrepancy between placing the leaf blade face up and face down remains unclear, it is extremely worthwhile to clarify the mechanisms.

Fig. 2 shows the effects of IAA on petiole bending in detached leaves of *B. calycinum* placed leaf blade face down in horizontal postion and kept vertically. Interestingly, the application of IAA (0.1%, w/w) to the lower side of petioles substantially inihibited bending of the petiole kept not only in

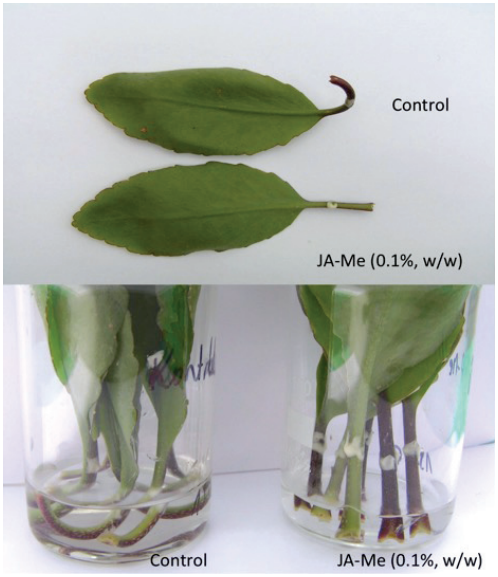

Fig. 1. Petiole bending in detached leaves of *B. calycinum*. Clear petiole bending was observed in detached leaves being placed leaf blade face down (upper) and kept in vertical position (lower). The application of JA-Me (0.1%, w/w) inhibited almost completely petiole bending in detached leaves. Pictures were taken 3 days after treatment.

horizontal but also in vertical positions, although application of IAA to the upper side or around of petiole did not affect it.

Bending of the excised petiole segments with or without partial leaf blade was also investigated. Detailed explanations of chemical treatments in these explants are described in Table 1. As shown in Fig. 3, relatively weak petiole bending in the excised petiole segments was observed, compared to bending in the petiole with the leaf blade. Partially remaining leaf blade also induced petiole bending to a significant extent. In addition, application of IAA as a lanolin paste to the middle of the excised petiole segments substantially induced strong petiole bending. Promoting effect of IAA on the epinastic curvature in isolated petioles of *Coleus* has been found (Soekarjo, 1965). These facts strongly suggest that adequate and/or suitable endogenous levels of auxin biosynthesized and/or produced in the leaf blade and transported to the petiole are essential to induce petiole bending in detached leaves of *B. calycinum*.

Ethephon was not effective to induce petiole bending, strongly suggesting that ethylene derived from exogenously applied IAA does not play an important role in petiole bending in detached leaves of *B. calycinum* (Fig. 4). This result confirms the previous observations reported by Ueda et al. (2018).

As shown in Figs. 1 and 4, relatively lower concentration of JA-Me was substantially effective to inhibit petiole bending in detached leaves as it was extremely effective to inhibit IAA-induced epinasty and/or hyponasty in intact *B. calycinum*, although its higher concentrations were not effective but the leaves finally withered and died, as already reported (Ueda et al., 2018). This discrepancy might be generally explained by a dose-response regulation of plant growth regulators. As already indicated by Ueda et al. (2018), the mode of action of JA-Me to inhibit petiole bending in detached leaves has not been clarified yet, but it might be related to its inhibitory effect on polar auxin transport as already suggested (Sun et al., 2009, 2011; Hentrich et al., 2013).

Loeb (1917) intensively studied the influence of the leaf upon root formation and geotropic (gravitropic) curvature in the stem of *B. calycinum*, claiming that extremely strong stem bending was observed when a stem segment in apcal side with

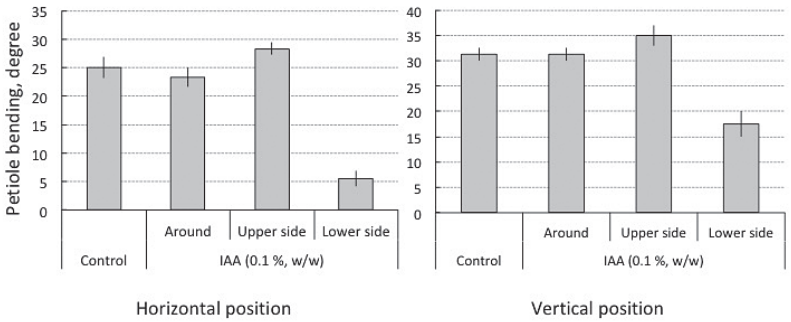

Fig. 2. Effect of IAA on petiole bending in detached leaves of *B. calycinum* placed leaf blade face down in horizontal position and kept vertically. IAA (0.1%, w/w) in lanolin paste was applied to the upper, the lower or around petioles in detached leaves placed leaf blade face down. Measurements were made 3 days after treatment. Data were expressed as average values with standard error of the mean in each treatment (n = 3 to 5).

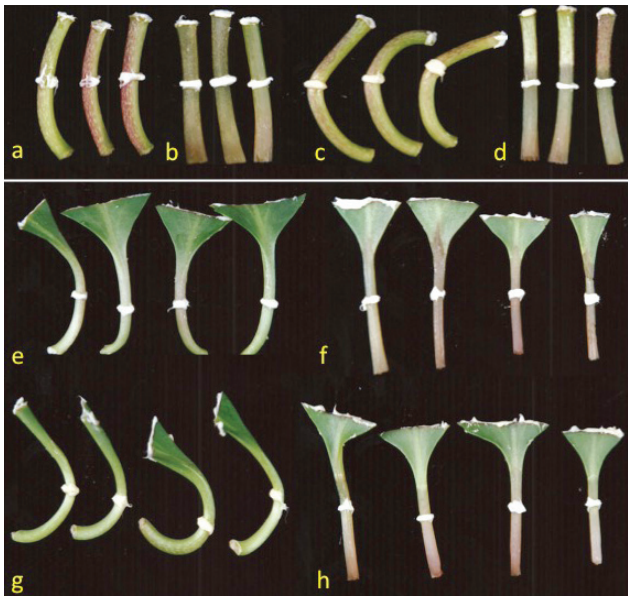

Fig. 3. Bending of excised petiole segments without (a to d) or with (e to h) partial leaf blade of *B. calycinum*. Detailed explanation of each treatment is indicated in Table 1. Pictures (a to d) and (e to h) were taken 6 days and 10 days after treatment, respectively.

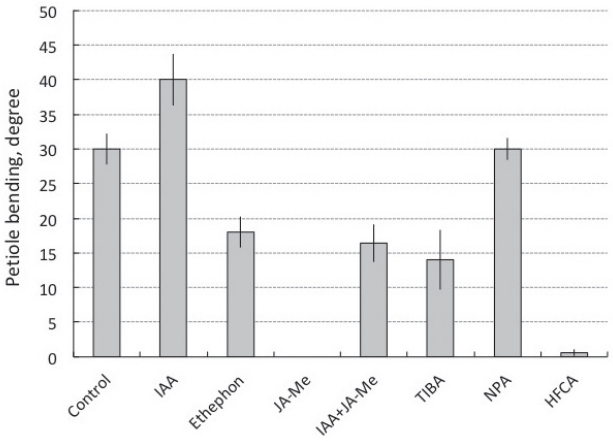

Fig. 4. Effects of IAA, ethephon, JA-Me and inhibitors of polar auxin transport (TIBA, NPA and HFCA) on petiole bending in detached leaves of *B. calycinum*. IAA (0.1%, w/w), ethephon (1%, w/w), JA-Me (0.1%, w/w), TIBA (0.2%, w/w), NPA (0.2%, w/w) and HFCA (0.2%, w/w) in lanolin paste were applied to almost middle part of petioles in detached leaves. Detached leaves were placed leaf blade face down in horizontal position. Measurements were made 3 days after treatment. Data were expressed as average values with standard error of the mean in each treatment (n = 5).

leaf was horizontally placed but almost no stem bending was found when a stem segment in basal side with leaf was placed horizontally. In addition, clear stem bending was observed when half of the stem was horizontally placed in the cut surface above. However, almost no stem bending was found when half of the stem was horizontally placed in the cut surface below. Based on these results, it was suggested that the tension affected by unknown factors from leaves between cortex (epidermis with a few cell layers of the inner tissue) and the inner tissue are responsible for stem bending. One of the unknown factors has been recognized as endogenous auxin. Recently, a biochemical and molecular review of the phenomenon of epinasty, and the role of auxin in these processes have been described by Sandalio et al. (2016). It has been suggested that the homeostatic level of IAA plays an important role in regulation of the balance between adaxial and abaxial cell growth in leaves, and epinasty is associated with accumulation of auxin on the adaxial side, causing increased growth of the adaxial surface, as compared to the abaxial one. Similar explanation might be possible for petiole bending in detached leaves of *B. calycinum*. Relatively high amount of IAA applied to petioles of *B. calycinum* seems to change the balance of auxin graduations, resulting in different elongation of the upper and the lower sides of the petiole and, consequently, inducing petiole bending when the detahced leaves were placed leaf blade face down. In detached leaves of *B. calycinum*, not only endogenous IAA from the leaf blade but also exogenously applied one might induce differential growth between the upper and the lower sides of the petiole.

Potent inhibitors of polar auxin transport, TIBA and HFCA were extremely effective to inhibit petiole bending (Fig. 4). Since extremely small bending was observed in the excised petiole segment without the leaf blade (Fig. 3), these results strongly support the above suggestions that transport and/or movement of endogenous auxin produced in the leaf blade are necessary to induce petiole bending in detached leaves of *B. calycinum*, although NPA had no effect on petiole bending (Fig. 4). Quite similar observations have been found in rooting of *Bryophyllum calycinum*, *B. daigremontianum*, *Kalanchoe blossfeldiana* and *K. tubiflora* induced by inhibitors of polar auxin transport. TIBA and morphactin IT 3456, whose chemical structure resembles HFCA, completely inhibited rooting but NPA did not (Saniewski et al., 2014; Ueda et al., 2016).

The mode of action of NPA, TIBA and HFCA in inhibiting polar auxin transport is really controversial. In the study which used the *pis1* mutant of *Arabidopsis*, NPA, TIBA and HFCA have been reported to represent different classes of auxin transport inhibitors. The *pis1* mutation conferred hypersensitivity to both NPA and TIBA

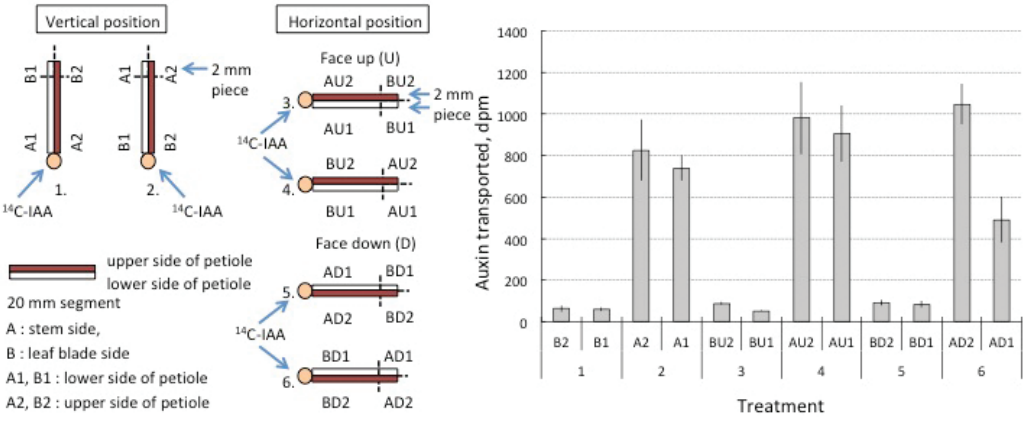

Fig. 5. Experimental design (left) and polar auxin transport (right) in excised petiole segments of *B. calycinum*. Polar auxin transport was clearly observed in excised petiole segments, radiolabeled auxin being substantially transported only from the leaf blade side of petiole to the stem one. When detached leaves were placed leaf blade face down, accumulation of radiolabled IAA was extremely reduced in the lower side of the excised petiole segments (AD1 in Treatment 6). Data were expressed as average values with standard error of the mean in each treatment (n = 5).

but not to HFCA, suggesting that the PIS1 protein is specifically involved in the response pathway of NPA and/or TIBA (Fujita and Syono, 1997). As described above, it has been reported that TIBA and morphactin IT 3456 inhibited root formation in the cuttings of some species of Crassulaceae when these inhibitors of auxin transport were applied around the stem below the leaves, but did not when these inhibitors were applied around the stem near the top and leaves were present below the treatment. NPA induced conspicuous local internode swelling only in the area of its application to *B. calycinum*, but TIBA did not (Saniewski et al., 2014, 2017; Ueda et al., 2016). Root emergence and root development from epiphyllous plantlets on the excised leaves of *Bryophyllum marnierianum* were completely inhibited by application of TIBA, whereas NPA had almost no effect on plantlet root development on leaves (Kulka, 2008). Moreover, NPA has been reported not to affect the formation of vegetative or floral structures at bracteoles, which are formed after removal of flower buds in CAM (crassulacean acid metabolism) plants of *Agave tequilana* (Abraham et al., 2016). Unlike TIBA, NPA almost did not inhibit rhizogenesis in hypocotyl of CAM plants of *Mesembryanthemum crystallinum* (Konieczny et al., 2009). TIBA increased root bud growth in CAM plants of *Euphorbia escula* (Nissen and Foley, 1987), but NPA had little effect on the growth of the root buds (Horvath, 1998). This apparent discrepancy in the mode of action of these inhibitors could be explained by the different interaction with different proteins relevant to polar auxin transport as already suggested (Saniewski et al., 2014; Ueda et al., 2016). Judging from the results of the study for transcript, protein and metabolite temporal dynamics in the CAM plants of *Agave* (Abraham et al., 2016) together with the fact that CAM plants differ from C3 plants in the mechanisms of photosynthesis, it could not be excluded that NPA binding and/or associated proteins in *B. calycinum* are not the same as those in C3 plants, such as *Arabidopsis*.

TRANSPORT AND/OR MOVEMENT OF RADIOLABELED AUXIN IN EXCISED PETIOLE SEGMENTS OF *BRYOPHYLLUM CALYCINUM*

To clarify the polar transport and the distribution of auxin in petiole segments of *B. calycinum*, the accumulation of radiolabeled auxin in the excised petiole segments placed horizontally face up and face down was determined using [1-^{14}C] IAA (Figs. 5 and 6). As shown in Fig. 5, polar auxin transport was clearly observed in the excised petiole segments, radiolabled IAA being substantially transported only from the leaf blade side of the petiole to the stem side. This finding was almost the same as the results already reported for other plants (Osborne et al., 1969; Veen et al., 1969). In addition, the enhancement of polar auxin transport was found when auxin was applied to the cut surface of the lower side of petioles, compared with the case when auxin was applied to that of the upper one. The reason has not been clear yet but it might be proposed based on the results of microscopic observations. The distribution of phloem, and its related vascular and parenchymatous tissues which are considered to be responsible for polar auxin transport were observed only in the lower side of

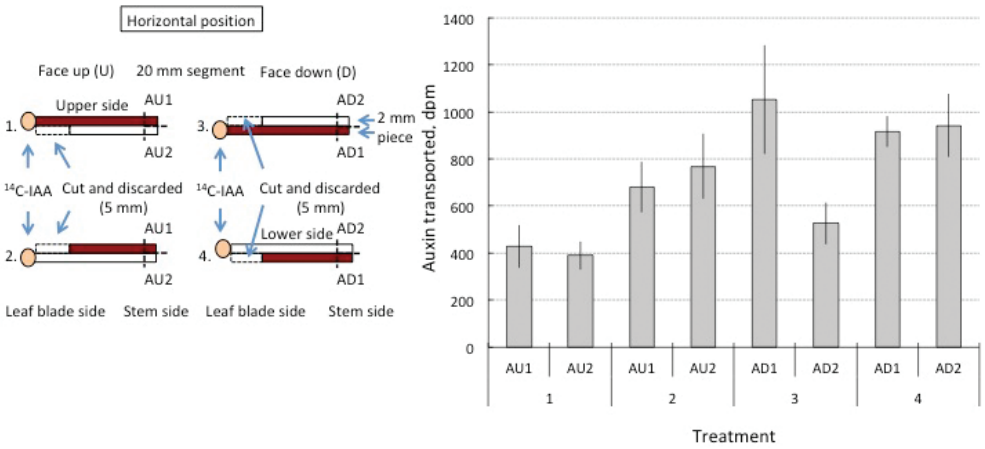

Fig. 6. Experimental design (left) and polar transport and/or lateral movement of auxin (right) in excised petiole segments of *B. calycinum*. Significant decrease of radiolabeled auxin in the lower side of petiole was observed when petiole segments were placed face down (AD2 in Treatment 3). Data were expressed as average values with standard error of the mean in each treatment (n = 5).

the petiole but not in the upper one in *B. calycinum* (data not shown).The microscopic observations in this study were quite similar to those made by Sharma and Naresh (2014). When detached leaves were placed leaf blade face down, accumulation of radiolabled IAA was extremely reduced in the lower side of the excised petiole segments (Fig. 5, AD1 in Treatment 6). This fact suggests that auxin in the lower side of the excised petiole segments is transported and/or moved laterally to the direction of gravity. Transverse distributions of auxin in geotropically (gravitropically) exposed pea roots (Konings, 1967) and in gravistimulated pea epicotyls (Hoshino et al., 2006) have already been reported. These results strongly suggest that polar transport and/or lateral movement of endogenous auxin biosynthesized and/or produced in the leaf blade are necessary to induce petiole bending in detached leaves of *B. calycinum* and that the alteration of transport and/or lateral movement of auxin contributes to the asymmetric distribution of auxin, resulting in petiole bending. This hypothesis is strongly supported by the fact that application of IAA only to the lower side of petioles in detached leaves extremely reduced petiole bending when the detached leaves were placed not only leaf blade face down but also kept in vertical position as shown in Fig. 1. Since petiole bending in detached leaves was clearly observed when they were kept vertically (Fig. 1) and polar auxin transport was not affected in vertical position of the detached leaves (Fig. 5), it could not be excluded that the upper side of the petiole is more susceptible and/or responsive to IAA than the lower side in detached leaves of *B. calycinum*.

Significant radioactivities in the upper and the lower sides in the excised petiole segments were found when the isotope was taken from only the cut surface of the upper and the lower sides of the petioles, respectively, when the excised petioles were placed face down. Interestingly, in this case, the radioactivity in the upper side of the excised petiole segment was almost the same as that in the lower one when the excised petiole segments were placed face down. On the other hand, auxin distribution in the lower side of petiole was significantly reduced to ca. 50% of that in the upper side when the petiole segments were placed face down, resulting in asymmetrical distribution of auxin in the upper and the lower sides of the petioles (Fig. 6, AD2 in Treatment 3). This fact is considered to be the reason why strong bending in detached leaves of *B. calycinum* was induced when they were placed leaf blade face down. These results also suggest that lateral movement and consequent differential distribution of auxin are substantially suppressed by the case where the excised petioles were placed face down. To verify this hypothesis, determination of endogenous levels of auxin in the upper and the lower sides of petioles are now in progress using a gas-liquid chromatography-mass spectrometry (GC-MS) system with deuterium labeled IAA (d_5-IAA) as the internal standard according to the method already reported (Ueda et al., 2014).

Finally, the important question arises why the altered and/or changed auxin transport system described here is observed only when detached leaves are placed leaf blade face down. The reason and/or the mechanism have not been explained yet but it might be possible to indicate

that gravistimulation induces rapid alteration in the transport system of auxin in petioles of *B. calycinum*, especially in detached leaves. The assumption that the alteration in one-way lateral auxin distribution from the upper side to the lower sides of the excised petiole occurs due to a change in the direction of gravistimulation might not be excluded. Gravitropic response of *Kalanchoe* stems has been studied, revealing that there is a redistribution of cell elongation potential to the lower side of the gravistimulated stem but there is no evidence for overall enhancement of cell expansion in response to gravity (Meicenheimer and Nackid, 1994). The important involvement of calcium in lateral auxin transport during ethylene-induced epinasty in tomato (*Lycopersicon esculentum* Mill.) seedlings has been studied, concluding that gravity-insensitive, ethylene-induced Ca^{2+} redistribution and accumulation toward the abaxial side are closely coupled with the adaxial auxin redistribution/accumulation and, in turn, to the petiolar epinasty (Lee et al., 2008). Judging from this fact together with the results of this study, endogenous levels of calcium in petiole cells of *B. calycinum* should be determined to clarify the mode of action of auxin inducing gravity-responding epinasty and/or hyponasty of this plant.

Recent analyses of *Arabidopsis* indicate that the enhancement of tropic bending observed in the *mdr/pgp* mutant resulted from decreasing longitudinal auxin transport, and a consequent increase of lateral auxin flux (Noh et al., 2001, 2003). A similar hypothesis could explain the results of the present study. Disruption of the normal polar auxin transport in the upper side of the petiole may induce a one-way lateral auxin flow to the lower side against gravistimulation, resulting in petiole bending when detached leaves were only placed leaf blade face down. Since gravistimulated alterations of PIN proteins, whose functions are responsible for regulating polar auxin transport, have recently been suggested (Kamada et al., 2018a, 2018b), immunohistochemical analyses using antibody of PIN proteins will be required in near future. Valuable findings of this study described here will be helpful to understand epinasty and/or hyponasty in *B. calycinum* adequately.

AUTHORS' CONTRIBUTIONS

The idea of the experiments: J.U., M.S.; performing the experiments: J.U., K.M., J.G.-K., M.S.; writing the manuscript: J.U., M.S., K.M.; invaluable discussions: J.U., M.S., K.M. The authors declare that they have no conflict of interest.

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REFERENCES

Abraham PE, Yin H, Borland AM, Weighill D, Lim SD, Cestari De Paoli H, Engle N, Jones PC, Agh R, Weston DJ, Wullschleger SD, Tschaplinski T, Jacobson D, Cushman JC, Hettich RL, Tuskan GA, and Yang X. 2016. Transcript, protein and metabolite temporal dynamics in the CAM plant *Agave*. *Nature Plants* 2: 16178.

Amrhein N, and Schneebeck D. 1980. Prevention of auxin-induced epinasty by α-aminooxyacetic acid. *Physiologia Plantarum* 49: 62–64.

Daniel SG, and Rayle DL. 1988. Ethylene modifies auxin transport in the auxin-insensitive tomato mutant, diageotropica. Abstracts, 13th International Conference on Plant Growth Substances, Calgary, Canada (July 17–22), p. 308.

Engelmann W, Sommerkamp A, Veit S, and Hans J. 1997. Methyl-jasmonate effects the circadian petal movement of *Kalanchoe* flowers. *Biological Rhythm Research* 28: 377–390.

Fujita H, and Syono K. 1997. PIS1, a negative regulator of the action of auxin transport inhibitors in *Arabidopsis thaliana*. *The Plant Journal* 12: 583–595.

Harvey EM. 1915. Some effects of ethylene on the metabolism of plants. *Botanical Gazette* 60: 193–204.

Hentrich M, Böttcher C, Düchting P, Cheng Y, Zhao Y, Berkowitz O, Masle J, Medina J, and Pollmann S. 2013. The jasmonic acid signaling pathway is linked to auxin homeostasis through the modulation of *YUCCA8* and *YUCCA9* gene expression. *The Plant Journal* 74: 626–637.

Horvath DP. 1998. The role of specific plant organs and polar auxin transport in correlative inhibition of leafy spurge (*Euphorbia escula*) root buds. *Canadian Journal of Botany* 76: 1227–123.

Hoshino T, Miyamoto K, and Ueda J. 2006. Requirement for the gravity-controlled transport of auxin for a negative gravitropic response of epicotyls in the early growth stage of etiolated pea seedlings. *Plant and Cell Physiology* 47: 1496–1508.

Kamada M, Miyamoto K, Oka M, Ueda J, and Higashibata A. 2018a. Regulation of asymmetric polar auxin transport by PsPIN1 in endodermal tissues of etiolated *Pisum sativum* epicotyls: focus on immunohistochemical analyses. *Journal of Plant Research* 131: 681–692.

Kamada M, Miyamoto K, Oka M, Uheda E, Ueda J, and Higashibata A. 2018b. Procedures for chemical fixation in immunohistochemical analyses of PIN proteins regulating polar auxin transport: Relevance to spaceflight experiments. *Life Sciences in Space Research* 18: 42–51.

Kazemi S, and Kefford NP. 1974. Apical correlative effects in leaf epinasty of tomato. *Plant Physiology* 54: 512–519.

Konieczny R, Kępczyński J, Pilarska M, Cembrowska D, Menzel D, and Samaj J. 2009. Cytokinin and ethylene affect auxin transport-dependent rhizogenesis in hypocotyl of com-

mon ice plant (*Mesembryanthemum crystallinum* L.). *Journal of Plant Growth Regulation* 28: 331–340.

Konings H. 1967. On the mechanism of the transverse distribution of auxin in geotropical exposed pea roots. *Acta Botanica Neerlandica* 16: 161–76.

Kulka RG. 2008. Hormonal control of root development on epiphyllous plantlets of *Bryophyllum* (*Kalanchoe*) *marnierianum*: role of auxin and ethylene. *Journal of Experimental Botany* 59: 2361–2370.

Leather GR, Forrence LE, and Abeles FB. 1972. Increased ethylene production during clinostat experiments may cause leaf epinasty. *Plant Physiology* 49: 183–186.

Lee Y, Jung J-W, Kim S-K, Hwang Y-S, Lee J-S, and Kim S-H. 2008. Ethylene-induced opposite redistributions of calcium and auxin are essential components in the development of tomato petiolar epinastic curvature. *Plant Physiology and Biochemistry* 46: 685–693.

Loeb J. 1917. Influence of the leaf upon root formation and geotropic curvature in the stem of *Bryophyllum calycinum* and the possibility of a hormone theory of these processes. *Botanical Gazette* 63: 25–50.

Meicenheimer RD, and Nackid TA. 1994. Gravitropic response of *Kalanchoe* stems. *International Journal of Plant Sciences* 155: 395–404.

Nissen SJ, and Foley ME. 1987. Correlative inhibition and dormancy in root buds of leafy spurge (*Euphorbia esc*ula). *Weed Science* 35: 155–159.

Noh B, Bandyopadhyay A, Peer WA, Spalding EP, and Murphy AS. 2003. Enhanced gravi- and phototropism in plant *mdr* mutants mislocalizing the auxin efflux protein PIN1. *Nature* 423: 999–1002.

Noh B, Murphy AS, and Spalding EP. 2001. Multidrug Resistance-like genes of *Arabidopsis* required for auxin transport and auxin-mediated development. *The Plant Cell* 13: 2441–2454.

Osborne DJ. 1982. The ethylene regulation of cell growth in specific target tissues of plants. In: PF Wareing, ed, *Plant Growth Substances*, 279–290. Academic Press, New York.

Osborne DJ, and Mullins MG. 1969. Auxin, ethylene and kinetin in a carrier protein model system for the polar transport of auxins in petiole segments of *Phaseolus vulgaris*. *New Phytologist* 68: 977–991.

Polko JK, van Zanten M, van Rooij JA, Marée AFM, Laurentius ACJ, Voesenek ACJL, Anton J. M. Peeters AJM, and Pierik R. 2012. Ethylene-induced differential petiole growth in *Arabidopsis thaliana* involves local microtubule reorientation and cell expansion. *New Phytologist* 193: 339–348.

Saltveit, ME, Pharr DM, and Larson RA. 1979. Mechanical stress induces ethylene production and epinasty in poinsettia cultivars. *Journal of American Society for Horticultural Science* 104: 452–455.

Saltveit ME, and Larson RA. 1983. Effect of mechanical stress and inhibitors of protein synthesis on leaf epinasty in mechanically stressed poinsettia plants. *Journal of American Society for Horticultural Science* 108: 253–257.

Sandalio LM, Rodriguez-Serrano M, and Romero-Puertas MC. 2016. Leaf epinasty and auxin: A biochemical and molecular overview. *Plant Science* 253: 187–193.

Saniewski M, Góraj J, Węgrzynowicz-Lesiak E, Miyamoto K, and Ueda J. 2014. Differential effects of auxin polar transport inhibitors on rooting in some Crassulaceae species. *Acta Agrobotanica* 67: 85–92.

Saniewski M, Góraj-Koniarska J, Gabryszewska E, Miyamoto K, and Ueda J. 2017. Differential effects of *N*-1-naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) on auxin control of swelling of the shoots of *Bryophyllum calycinum* Salisb. *Acta Agrobotanica* 70: 1723. doi.org/10.5586/aa.1723

Schlagnhaufer CD, and Arteca RN. 1985. Brassinosteroid-induced epinasty in tomato plants. *Plant Physiology* 78: 300–303.

Sharma AJ, and Naresh C. 2014. Pharmacognostical studies of *Bryophyllum pinnatum* (Lam.) Kurz. *Pharmacognosy Journa*l 6: 20–26.

Soekarjo R. 1965. On the formation of adventitious roots in cuttings of *Coleus* in relation to the effect of indoleacetic acid on the epinastic curvature of isolated petioles. *Acta Botanica Neerlandica* 14: 373–399.

Sun J, Chen O, Qi L, Jiang H, Li S, Xu Y, Liu F, Zhou W, Pan J, Li X, Palme K, and Li C. 2011. Jasmonate modulates endocytosis and plasma membrane accumulation of the *Arabidopsis* PIN2 protein. *New Phytologist* 191: 360–375.

Sun J, Xu Y, Ye S, Jiang H, Chen O, Liu F, Zhou W, Chen R, Li X, Tietz O, Wu X, Cohen JD, Palme K, and Li C. 2009. *Arabidopsis ASA1* is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. *The Plant Cell* 21: 1495–1511.

Ueda J, Góraj-Koniarska J, Miyamoto K, and Saniewski M. 2018. Epinasty and/or hyponasty, and petiole growth in *Bryophyllum calycinum*: Focus on the interaction of indole-3-acetic acid and methyl jasmonate. *Acta Biologica Cracoviensia Series Botanica* 60: 73–81.

Ueda J, Miyamoto K, Uheda E, Oka M, Yano S, Higashibata A, and Ishioka N. 2014. Close relationships between polar auxin transport and graviresponse in plants. *Plant Biology* 16: 43–49.

Ueda J, Miyamoto K, Yuda T, Hoshino T, Fujii S, Mukai C, Kamigaichi S, Aizawa S, Yoshizaki I, Shimazu T, and Fukui K. 1999. Growth and development, and auxin polar transport in higher plants under microgravity conditions in space: BRIC-AUX on STS-95 space experiment. *Journal of Plant Research* 112: 487–492.

Ueda J, Miyamoto K, Yuda T, Hoshino T, Sato K, Fujii S, Kamigaichi S, Izumi R, Ishioka N, Aizawa S, Yoshizaki I, Shimazu T, and Fukui K. 2000. STS-95 space experiment for plant growth and development, and auxin polar transport. *Biological Sciences in Space* 14: 47–57.

Ueda J, Saniewski M, and Miyamoto K. 2016. Auxin, one major plant hormone, in soil. In: LW Szajdak ed, *Bioactive Compounds in Agricultural Soils*, 175–209. Springer-Verlag. ISBN 978-3-319-43106-2

Ueda J, Toda Y, Kato K, Kuroda Y, Arai T, Hasegawa T, Shigemori H, Hasegawa K, Kitagawa J, Miyamoto K, and Uheda E. 2013. Identification of dehydrocostus lactone and 4-hydroxy-b-thujone as auxin polar transport inhibitors. *Acta Physiologiae Plantarum* 35: 2251–2258.

Veen H, and Jacobs WP. 1969. Transport and metabolism of indole-3-acetic acid in *Coleus* petiole segments of increasing age. *Plant Physiology* 44: 1157–1162.