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EPINASTY AND/OR HYPONASTY, AND PETIOLE GROWTH IN *BRYOPHYLLUM CALYCINUM*: FOCUS ON THE INTERACTION OF INDOLE-3-ACETIC ACID AND METHYL JASMONATE

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

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During the research interaction of indole-3-acetic acid (IAA) and methyl jasmonate (JA-Me) in epinasty and/or hyponasty, as well as petiole growth of *Bryophyllum calycinum* were investigated. Exogenously applied IAA as a lanolin paste was extremely effective to induce epinasty and/or hyponasty accompanied with petiole elongation in intact *B. calycinum*. Application of IAA around or to the upper side of the petiole was much more effective than that to the lower side, suggesting that petiole epidermal cells on the adaxial side of *B. calycinum* are more sensitive and/or susceptive to IAA than those on the abaxial one. This is supported by the fact that not only the second curvature but also the first one in *B. calycinum* was enhanced by application of IAA to the upper side of the petiole. The degree of epinasty and/or hyponasty induced by IAA is strongly related to the increase of petiole growth. On the other hand, JA-Me significantly inhibited IAA-inducing epinasty and/or hyponasty, and petiole growth in intact *B. calycinum*. When detached leaves with petioles were placed leaf blade face down, clear petiole bending was observed. However, no petiole bending was found when detached leaves were placed leaf blade face up. Exogenously applied IAA to petioles was significantly effective to induce and/or stimulate petiole bending in placing detached leaves of *B. calycinum* face down but ethephon was not, suggesting 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* and that ethylene derived from exogenously applied IAA does not play an important role in epinasty and/or hyponasty, and petiole bending in *B. calycinum*. The mechanisms of IAA-enhancing and JA-Me-inhibiting epinasty and/or hyponasty, and petiole growth are intensively discussed.

Keywords: auxin, *Bryophyllum calycinum*, epinasty, ethylene, hyponasty, indole-3-acetic acid, methyl jasmonate

INTRODUCTION

The posture of plants at their developmental stages is affected by surrounding environmental stimuli such as light, temperature, gaseous constituents, moisture status and so on. Plants substantially change their own growth and development in response to environmental stimuli. Epinasty and/or hyponasty are interesting phenomena, being one of perplexing behaviors found in many plants grown under unfavorable conditions. Epinasty in leaves with petioles is the most popular. The upper part of cells in the petiole outgrows the bottom ones and the leaf drops from a horizontal to a more vertical position. Epinasty and/or hyponasty have been well known to be caused by auxin and/or ethylene (Harvey, 1915; Kazemi and Kefford, 1974; Leather et al., 1972) but the mechanisms of these plant hormones in the induction of epinasty and/or hyponasty are still controversial. It has been suggested that homeostatic level of IAA plays an important role in regulation of the balance between

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adaxial and abaxial cell growth in leaves, and epinasty is associated with accumulation of auxin on the adaxial side, causing the increased growth of adaxial surface, as compared to the abaxial one (Sandalio et al., 2016).

Based on the data that an endogenous production of ethylene causes the epinastic response (Leather et al., 1972; Osborne, 1982), an important role of ethylene in petiole epinasty was proposed. It was also found that exogenously applied IAA and 2,4-dichlorophenoxyacetic acid (2,4-D) induced epinastic curvatures that increased with increasing concentrations in isolated petiole segments of *Coleus scutellariodes* (Soekarjo, 1965), which depended on endogenous production of ethylene derived from the applied auxin (Morgan and Hall, 1964). This hypothesis was supported by the following facts: (1) the partial reduction of epinasty was caused by the treatment with silver nitrate of a potent inhibitor of ethylene action (Saltveit Jr et al., 1979), (2) α-aminooxyacetic acid (AOA) and aminoethoxyvinylglycine (AVG) of inhibitors of endogenous production of ethylene (Amrhein and Schneebeck, 1980; Daniel and Rayle, 1988; Saltveit Jr and Larson, 1981) and (3) the epinasty of poinsettia (*Euphorbia pulcherrima* Willd.) was substantially induced by exogenous application of ethylene (Osborne, 1982).

The adaxial petiole cells responsible for epinastic growth are so-called type III target cells responding to ethylene but their growth is dependent upon the presence of auxin (Osborne, 1982). In some plants, type III cells also responded directly to auxin, but the simultaneous application of auxin and ethylene showed an additive response (Stange and Osborne, 1988). Removal of the bract blades prevented the epinastic response of the petiole, and the response was restored by applying IAA to the cut surface of the petiole end. Redistribution of auxin appears to be responsible for epinasty and/or hyponasty, and the increased ethylene production of reoriented poinsettia bracts (Michael et al., 1981). In tomato plants, the epinastic response of excised petiole sections was approximately log-linear concentrations of IAA and 2,4-D. When ethylene synthesis was inhibited by AVG, epinasty was no longer induced by auxin, but could be restored by addition of ethylene gas (Virginia and Bradford, 1989).

Methyl jasmonate (JA-Me) widely distributed in the plant kingdom has been well known not only as a fragrant constituent of the essential oil but also a physiologically active plant growth regulator. It plays key roles as a new type of plant hormone-like growth regulator, elicitor and signal transducer (Murofushi et al., 1999; Saniewski et al., 2002). Up date reviews of jasmonates including molecular mechanisms have recently been published (Wasternack, 2007; Wasternack and Hause, 2013; Wasternack and Susheng, 2016). The interaction of IAA and jasmonate including JA-Me and jasmonic acid (JA) has been considered to be important in regulating plant growth and development. JA does not appear to interact directly with IAA but rather to inhibit some physiological processes required for IAA-induced cell elongation in oat coleoptile segments (Ueda et al., 1994, 1995). Leaf abscission promoted by JA-Me was also inhibited by IAA (Ueda et al., 1996). Jasmonates have also been well known to promote ethylene production in multiple plant tissues (Saniewski, 1995). Since epinasty seems to be regulated by ethylene as described above, jasmonates are also suggested to interact with ethylene derived from applied IAA.

Recently, it was found that exogenously applied IAA enhanced epinasty and/or hyponasty, and elongation growth of the petiole of intact *Bryophyllum calycinum* plants, and JA-Me significantly inhibited both processes. Here, we report epinasty and/or hyponasty of intact *B. calycinum* induced by IAA in the presence and/or absence of JA-Me. Petiole bending relevance to epinasty and/or hyponasty in detached leaves of *B. calycinum* is also described. The mechanisms of epinasty and/or hyponasty relevance to the effects of IAA and JA-Me are well discussed.

MATERIAL AND METHODS

Two to three month-old plants of *Bryophyllum calycinum* Salisb., propagated continuously during the whole year from epiphyllous buds arising in the marginal notches of the leaves, were used for Experiment I on intact plants and Experiment II on detached leaves.

EXPERIMENT I

Lanolin pastes containing indole-3-acetic acid (IAA, 0.1%, w/w) in the presence or absence of JA-Me (0.5%, w/w) were applied around the petiole and/or to the upper or the lower side of the petiole. The treated plants were incubated in a greenhouse under natural conditions from July to August in Skierniewice, Poland. The first and the second angles of the treated plants were measured 24 and 48 hours after treatment. The length of the petiole was measured at the end of the experiment, 40 days after treatment, and expressed as increase or decrease in length, compared to that of the control. The methods of application of a lanolin paste and measurements of the first and the second angles were illustrated in Fig. 1. Chemical treatments (Treatment No 1. to No 41.) in this study are categorized into A to E as described below.

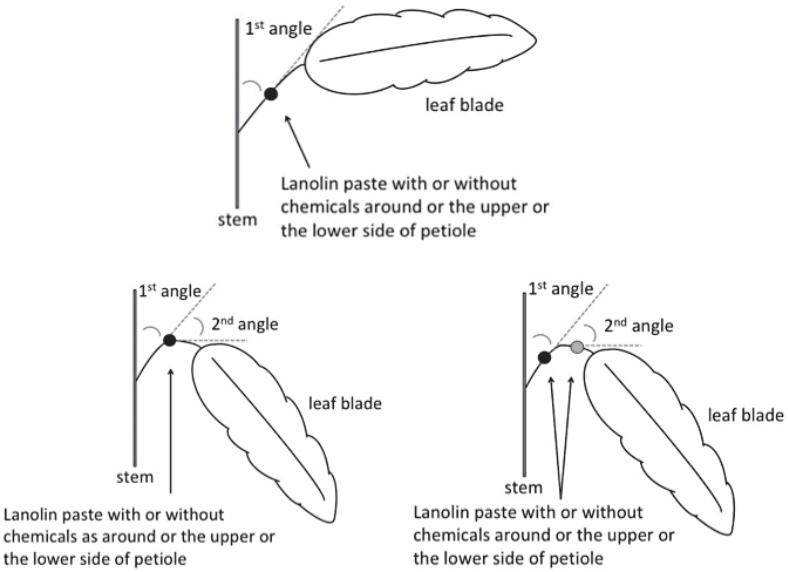

Fig. 1. Experimental design and application of chemicals (IAA, JA-Me, ethephon, NPA, TIBA and GA_3) as lanolin pastes to petioles of *Bryophyllum calycinum*. Details of chemical treatment and its applied positions are shown in Table 1.

(A) Lanolin pastes only or lanolin pastes containing IAA (0.1%, w/w) or JA-Me (0.5%, w/w), and mixture of IAA (0.1%, w/w) and JA-Me (0.5%, w/w) were applied around the petiole or all over the petiole (Treatments: 1 to 4 and 22).
(B) Lanolin pastes containing IAA (0.1%, w/w) or JA-Me (0.5%, w/w), and mixture of IAA (0.1%, w/w) and JA-Me (0.5%, w/w) were applied to the upper or lower side of the petioles (Treatments: 5 to 9 and 10).
(C) Lanolin pastes containing IAA (0.1%, w/w) or JA-Me (0.5%, w/w), and mixture of IAA (0.1%, w/w) and JA-Me (0.5%, w/w) were applied to two places of the petiole (Treatments: 11 to 14).
(D) Lanolin pastes containing ethephon (1%, w/w), NPA (0.2%, w/w), TIBA (0.2%, w/w) and GA_3 (1%, w/w) with or without IAA (0.1%, w/w) were applied in several ways (Treatments: 15 to 21 and 23 to 26).
(E) To confirm the effects of IAA (0.1%, w/w) and JA-Me (0.5%, w/w) on petiole growth, and epinasty/or hyponasty, additional experiments were planned (Treatments: 27 to 41). In these experiments, the petiole length was measured 3 days after treatment.

Details of each treatment (Treatment No 1. to No 41.) are described in Table 1. Five intact plants were used for each treatment. Since *Bryophyllum calycinum* is an opposite-leaved plant, one petiole with the leaf blade was the control (lanolin only) and the other one was treated with chemicals. Pictures of epinasty and/or hyponasty were made 3 days after treatment. All data were expressed as average values with the standard error of the mean.

TABLE 1. Chemical treatments and treatment position of intact plants of *Bryophyllum calycinum*.

Treatment No.	Treatment	Treatment position
1	Control, lanolin paste only	around petiole
2	IAA (0.1%, w/w)	around petiole
3	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	around petiole
4	JA-Me (0.5%, w/w)	around petiole
5	IAA (0.1%, w/w)	upper side of petiole
6	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	upper side of petiole

TABLE 1 – continued

Treatment No.	Treatment	Treatment position
7	JA-Me (0.5%, w/w)	upper side of petiole
8	IAA (0.1%, w/w)	lower side of petiole
9	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	lower side of petiole
10	JA-Me (0.5%, w/w)	lower side of petiole
11	IAA (0.1%, w/w) and JA-Me (0.5%, w/w)	around petiole near stem and near leaf blade, respectively
12	JA-Me (0.5%, w/w) and IAA (0.1%, w/w)	around petiole near stem and near leaf blade, respectively
13	IAA (0.1%, w/w) and JA-Me (0.5%, w/w)	upper sides of petiole near stem and near leaf blade, respectively
14	JA-Me (0.5%, w/w) and IAA (0.1%, w/w)	upper sides of petiole near stem and near leaf blade, respectively
15	Ethephon (1%, w/w)	around petiole
16	Ethephon (1%, w/w)	upper side of petiole
17	Ethephon (1%, w/w)	lower side of petiole
18	NPA (0.2%, w/w) and IAA (0.1%, w/w)	around petiole near stem and near leaf blade, respectively
19	TIBA (0.2%, w/w) and IAA (0.1%, w/w)	around petiole near stem and near leaf blade, respectively
20	NPA (0.2%, w/w)	around petiole
21	TIBA (0.2%, w/w)	around petiole
22	IAA (0.1%, w/w)	all over petiole
23	GA_3 (1%, w/w)	around petiole
24	GA_3 (1%, w/w)	upper side of petiole
25	GA_3 (1%, w/w)	lower side of petiole
26	GA_3 (1%, w/w)	all over petiole
27	Control, lanolin paste only	around petiole
28	IAA (0.1%, w/w)	around petiole
29	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	around petiole
30	IAA (0.1%, w/w)	around petiole just near leaf blade
31	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	around petiole just near leaf blade
32	IAA (0.1%, w/w)	lower side of leaf blade
33	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	lower side of leaf blade
34	IAA (0.1%, w/w)	lower side of petiole
35	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	lower side of petiole
36	IAA (0.1%, w/w)	upper side of petiole
37	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	upper side of petiole
38	IAA (0.1%, w/w)	lower side of petiole just near leaf blade
39	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	lower side of petiole just near leaf blade
40	IAA (0.1%, w/w)	upper side of petiole just near leaf blade
41	IAA (0.1%, w/w) + JA-Me (0.5%, w/w)	upper side of petiole just near leaf blade

EXPERIMENT II

Immature and mature detached leaves with petioles of *B. calycinum* were placed leaf blade face up (normal position) or face down (inverted position). Lanolin pastes containing IAA (0.1 and 0.5%, w/w), ethephon (1%, w/w) and JA-Me (0.05% to 0.5%, w/w) were applied to the middle part of petioles of detached leaves. The treated plants were kept for 76 hours in a tray with several layers of filter papers moistened with distilled water in a greenhouse under natural conditions, the same as those in Experiment I. Relative humidity of a tray for incubating treated plants was oversaturated. At the end of the experiments, curvatures in the treated plants were measured as shown in Fig. 2. The application method of the lanolin paste used in this study was also illustrated in Fig. 2. Five detached leaves were used for each treatment. Petioles of detached leaves with or without lanolin only were used as the control. Pictures of petiole bending were made 2 days after incubation. Data were expressed as average values with the standard error of the mean.

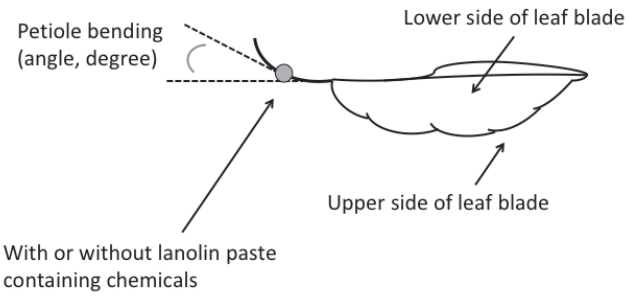

Fig. 2. Experimental design and application of IAA and ethephon as lanolin pastes to petioles in detached leaves of *Bryophyllum calycinum* placed leaf blade face down.

RESULTS AND DISCUSSION

It should be mentioned that experiments similar to those presented in this study were also performed in other months (January to May and September to December) and the epinasty and/or hyponasty of *B. calycinum* relevance to the effects of IAA and JA-Me were quite similar, suggesting that these physiological phenomena of this plant are independent of photoperiod, light intensity and temperature (about 18 to 24°C) in the greenhouse.

Epinasty and/or hyponasty of *B. calycinum* were investigated when IAA and JA-Me, and other chemicals were exogenously applied to petioles as lanolin pastes as illustrated in Fig. 1 and described in Table 1 in detail. As shown in Fig. 3, reactions of the petiole after treatment with IAA and mixture IAA + JA-Me depend on the positions of treatment: epinasty (Fig. 3a) and hyponasty (Fig. 3c) are clearly visible.

Figs. 4a and 4b show the first and the second angles of petioles of *B. calycinum* 24 hours after treatment, respectively. The first angle of the petioles in the treatment with IAA was almost the same as that of the control with or without the lanolin paste. When JA-Me with IAA were simultaneously applied around the petiole and the upper side of it, the first angle was slightly enhanced. On the contrary, application of IAA to the lower side of the petiole extremely reduced the first angle and JA-Me significantly inhibited it (Fig. 4a). The first and the second angles 48 hours after treatment were almost the same as those after 24 hours (data not shown), indicating that epinastic growth was stopped only 24 hours after treatment with IAA and JA-Me. These results reveal that epinastic growth of the petiole was extremely inhibited and enhanced by application of IAA and JA-Me, respectively. The second angle was extremely enhanced by application of IAA not only around the petiole but also to the upper side of it. When IAA was applied to the lower side of the petiole, almost no second angle was observed. These results strongly suggested that petiole cells on the adaxial side of *B. calycinum* are more sensitive and/or susceptive to IAA than those in the abaxial one. Simultaneous application of JA-Me with IAA significantly reduced the second angle. Application

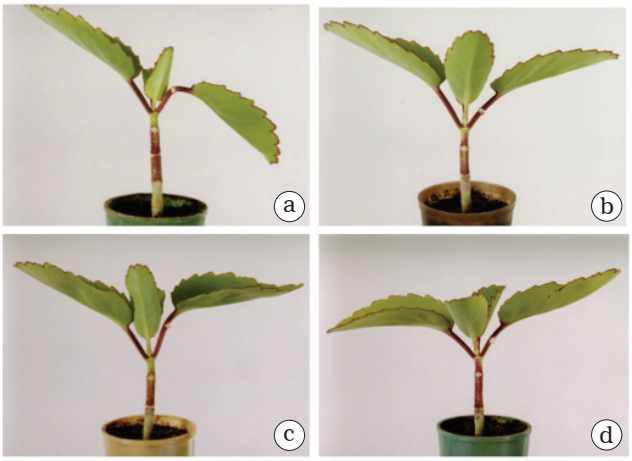

Fig. 3. Effects of IAA (0.1%) and mixture of IAA (0.1%) + JA-Me (0.5%) on epinasty and/or hyponasty curvature in petioles of *Bryophyllum calycinum* (typical examples of the treatment). (**a**) IAA (0.1%) applied in around the petiole. (**b**) IAA (0.1%) and JA-Me (0.5%) applied simultaneously in around the petiole. (**c**) IAA (0.1%) applied to the lower side of the petiole. (**d**) IAA (0.1%) and JA-Me (0.5%) applied simultaneously on the lower side of the petiole.

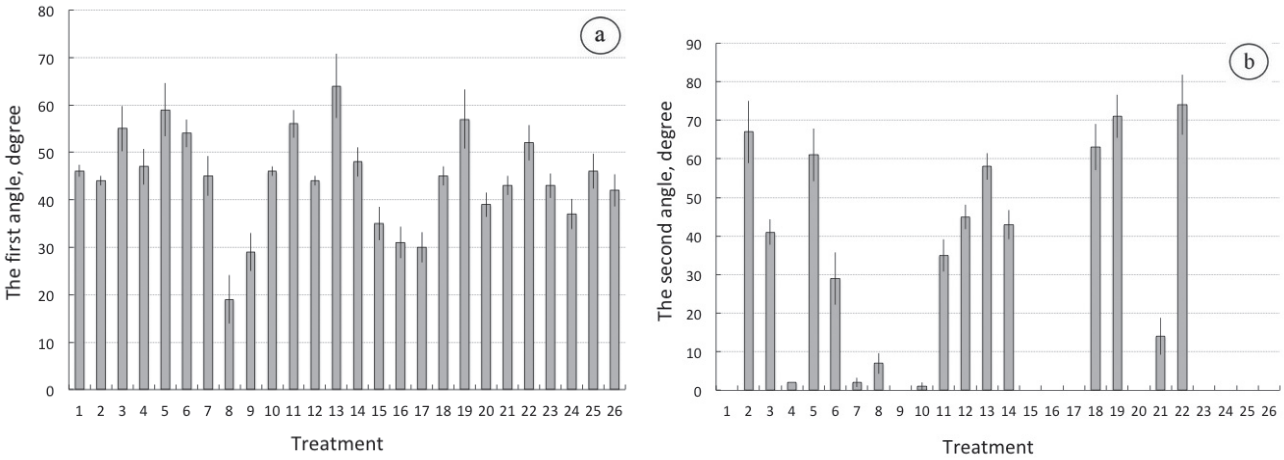

Fig. 4. The first and second angles of *Bryophyllum calycinum* affected by exogenously applied chemicals as lanolin pastes. Angles were measured as illustrated in Fig. 1. Details of chemical treatment and its applied positions are shown in Table 1. Measurements were made 24 hours after treatment. Data were expressed as the average value with the standard error of the mean (n = 5). (**a**) The first angle. (**b**) The second angle.

of JA-Me around or to the upper side of the petiole hardly induced the second angle (Fig. 4b).

Exogenous application of ethephon, NPA, and TIBA as lanolin pastes had almost no effect on the first angle in *B. calycinum* (Fig. 4a). The second angle was not affected by application of ethephon and NPA, but TIBA partially increased it (Fig. 4b). These results strongly suggest that ethylene and polar auxin transport are not directly effective in epinasty and/or hyponasty of this plant, although lateral transport of auxin was reported to be substantially effective in epinasty of leaves of *Coleus blumei*, *Euphorbia pulcherrima* and *Lycopersicon esculentum* (Lyon, 1963). Exogenously applied GA_3 was not effective to change the first and the second angles of *B. calycinum* (Figs. 4a and 4b), although a significant possible role of endogenous gibberellin in stem growth of *Bryophyllum* plants has been suggested (Šebánek et al., 1978).

When detached leaves with petioles were placed leaf blade face up, no petiole bending was found, even after 21 days of incubation. On the other hand, strong bending of petioles was observed when detached leaves with petioles were placed leaf blade face down; the petioles became convex on the lower side and concave on the upper side (Figs. 2 and 5). This discrepancy in petiole bending between leaf blade face up and face down remains unclear but it might be due to endogenous auxin dynamics. As already suggested by numerous researchers, auxin shows a tendency to accumulate on the lower side of a horizontally placed stem and of other plant organs (Firml et al., 2002; Forest et al, 2006; Konings, 1967). When detached leaves of *B. calycinum* are placed horizontally in the normal position (leaf blade face up), more auxin is accumulated on the lower (abaxial) side of the petiole which might be less sensitive for auxin, finally no bending was observed. On the other hand, when detached leaves are placed horizontally in the inverted position (leaf blade face down), again more auxin is accumulated on the lower (adaxial) side of the petiole, which might be very sensitive and/or susceptive to auxin, resulting in auxin-inducing and/or stimulating petiole bending in detached leaves of *B. calycinum* as shown in Fig. 6. Exogenously applied IAA (0.1%, w/w) was significantly effective in petiole bending but not in its higher concentration (0.5%, w/w). On the other hand, ethephon was not as effective in petiole bending as in epinasty and/or hyponasty of intact *B. calycinum*, supporting the hypothesis that ethylene does not play an important role in physiological phenomena in *B. calycinum*. Although

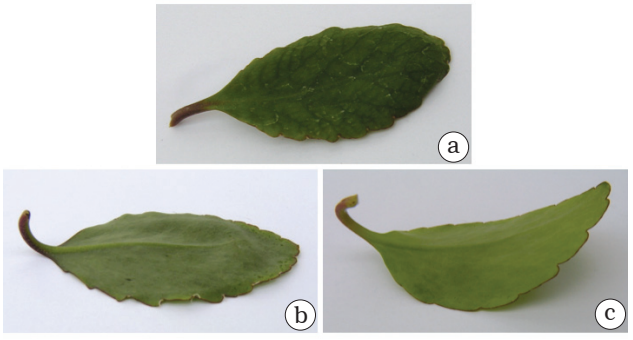

Fig. 5. Clear petiole bending in detached leaves of *Bryophyllum calycinum* when leaf blade was placed face up (**a**) and face down (**b** and **c**). Pictures were made 2 days after incubation.

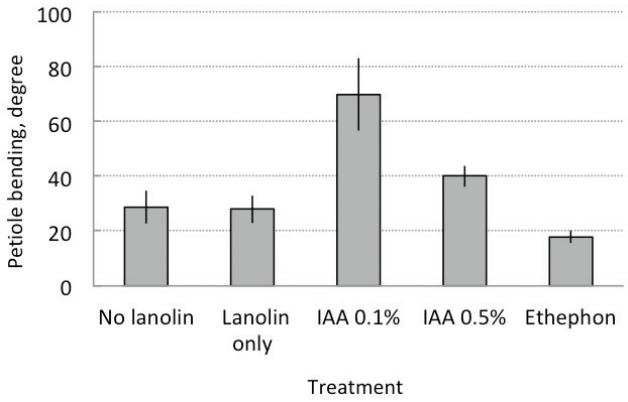

Fig. 6. Petiole bending in detached leaves of *Bryophyllum calycinum* affected by exogenously applied IAA (0.1% and 0.5%, w/w) and ethephon (1%, w/w) to the middle of petioles as a lanolin paste. Detached leaves were placed leaf blade face down. Petiole bending is expressed as angles measured as illustrated in Fig. 2. Measurements were made 76 hours after treatment. Data are expressed as the average value with the standard error of the mean (n = 5).

exogenously applied IAA has been well known to produce much of ethylene in numerous plants, exogenously applied IAA and ethephon substantially induced and/or stimulated, and significantly inhibited, respectively, petiole bending not only in intact plants but also in detached leaves of *B. calycinum* examined in this study. Sandalio et al. (2016) 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 the increased growth of the adaxial surface, as compared to the abaxial one. Similar explanation would be possible for epinasty and/or hyponasty in *B. calycinum* shown in the present study. Relatively high amount of IAA applied to petioles of *B. calycinum* might induce disturbance of auxin graduations.

JA-Me (0.05% to 0.5%, w/w) was almost no effective to induce petiole bending in detached leaves of *B. calycinum*, which finally withered and died (data not shown), although it was extremely effective to inhibit IAA-induced epinasty and/or hyponasty in intact *B. calycinum*. It probably depends on application doses of JA-Me compared to that of endogenous levels of auxin in detached leaves. Much lower concentrations of JA-Me might be effective to inhibit petiole bending in detached leaves of *B. calycinum*.

Figs. 7 and 8 show the effects of IAA and JA-Me on petiole growth in intact *B. calycinum*. The results were expressed as increase and/or decrease in petiole length, compared to that of the control (lanolin only). Exogenously applied IAA extremely promoted petiole growth and JA-Me significantly inhibited it 40 days (Fig. 7) and 3 days after treatment (Fig. 8). Potent inhibitors of polar auxin transport, TIBA and NPA, applied simultaneously with IAA did not inhibit petiole growth induced by IAA, but TIBA applied alone stimulated petiole elongation. Fujita and Syono (1996) suggest that TIBA itself has a weak auxin-like activity in *Arabidopsis thaliana*. Ethephon had almost no effect on petiole growth, although modification of polar auxin transport induced by ethylene has been reported (Beyer Jr and Morgan, 1971).

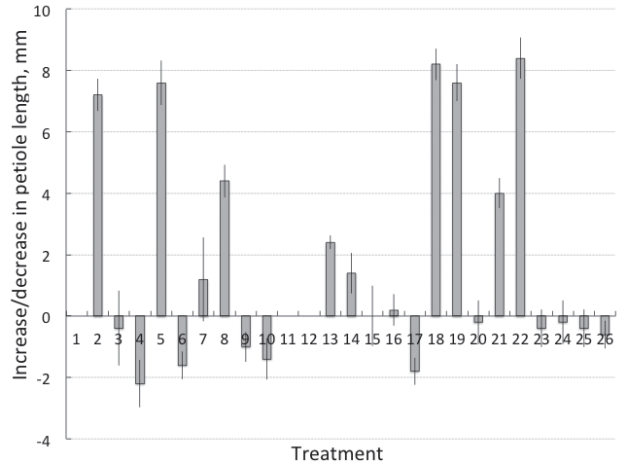

Fig. 7. Effects of exogenously applied chemicals as lanolin pastes on petiole growth of *Bryophyllum calycinum*. Details of chemical treatment and its applied positions are shown in Table 1. Measurements were made 40 days after treatment. Data were expressed as the average value with the standard error of the mean (n = 5).

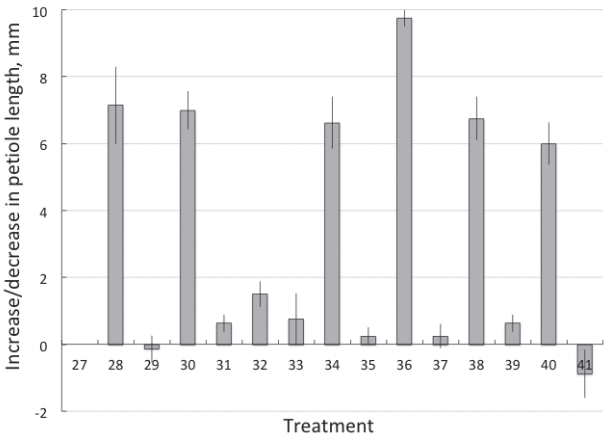

Fig. 8. Effects of exogenously applied IAA and JA-Me as lanolin pastes on petiole growth of *Bryophyllum calycinum*. Details of chemical treatment and its applied positions are shown in Table 1. Measurements were made 3 days after treatment. Data were expressed as the average value with the standard error of the mean (n = 5).

The mode of action of JA-Me to inhibit IAA-induced epinasty and/or hyponasty in *B. calycinum* has not been clarified yet. As described in Introduction, endogenously and/or exogenously applied ethylene has been well known to induce epinasty in many species of plants. In *B. calycinum*, exogenously applied ethephon had almost no effect on inducing epinasty and/or hyponasty, although exogenously applied IAA was extremely effective. Exogenously applied auxin and jasmonates have been well known to induce ethylene but in this case such ethylene might be rather ineffective. Judging from these results, epinasty and/or hyponasty, and petiole growth in *B. calycinum* induced by exogenously applied auxin or jasmonates do not depend on ethylene derived from these plant hormones. Exogenously applied jasmonate might affect auxin dynamics in *B. calycinum*. Evidence for a close functional relationship between jasmonates signaling pathway and auxin homeostasis has recently been documented (Hentrich et al., 2013; Pazmiño et al., 2014; Pérez and Goossens, 2013).

The role of reactive oxygen species (ROS) and nitric oxide (NO) in the regulation of epinasty has recently been established. ROS accumulation induced by auxins and 2,4-D has been found to trigger epinasty. Disturbances in the actin cytoskeleton induced 2,4-D through ROS and NO-dependent post-translational modifications in actin by carbonylation and S-nitrosylation have also been reported, suggesting that reorientation of microtubules is a major feature of the response to auxin and the cytoskeleton is therefore a key player in epinastic development (Sandalio et al., 2016). JA-Me has been well known to produce ROS when it caused activation of the programmed cell death (Zhang and Xing, 2008). The functional relationships between JA-Me and NO have also been investigated. As a result, JA-Me as well as ABA functions the downstream of the branch point in signal transduction pathways related to NO physiological phenomenon in *Arabidopsis* guard cells (Saito et al., 2009). Judging from these facts together with the results in this study, JA-Me might affect IAA-induced disturbance in the actin cytoskeleton through changing ROS and NO-dependent post-translational modifications in epinasty and/or hyponasty of *B. calycinum*. Promotive effect of 2,4-D in epinasty and/or hyponasty of *B. calycinum* has also been found in our experiments. JA-Me was extremely effective to inhibit 2,4-D inducing epinasty and/or hyponasty (data not shown). Further intensive investigations to clarify the mode of action of JA-Me will be significant, especially the relevance to its effect on ROS and NO-dependent post-translational modifications in actin.

AUTHORS' CONTRIBUTIONS

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

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