Acta Agrobotanica

DOI: 10.5586/aa.1660

Publication history
Received: 2015-11-20
Accepted: 2016-04-12
Published: 2016-05-18

Handling editor
Bożena Denisow, Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin, Poland

Authors' contributions
MS, JU: designed the experiment and wrote the manuscript; JGK, EG: carried out the experiments; KM: critically read the manuscript and contributed to data interpretation

Funding
The research was supported by the Polish Ministry of Science and Higher Education as part of the statutory activities (7.2.1) of the Department of General Biology, Research Institute of Horticulture in Skierniewice

Competing interests
MS, KM, JU are the members of the editorial council of the *Acta Agrobotanica*; other authors: no competing interests

Citation
Saniewski M, Góraj-Koniarska J, Gabryszewska E, Miyamoto K, Ueda J. Auxin effectively induces the formation of the secondary abscission zone in *Bryophyllum calycinum* Salisb. (Crassulaceae). Acta Agrobot. 2016;69(3):1660. http://dx.doi.org/10.5586/aa.1660

ORIGINAL RESEARCH PAPER

Auxin effectively induces the formation of the secondary abscission zone in *Bryophyllum calycinum* Salisb. (Crassulaceae)

Marian Saniewski[1]*, Justyna Góraj-Koniarska[1], Eleonora Gabryszewska[1], Kensuke Miyamoto[2], Junichi Ueda[3]

[1] Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland
[2] Faculty of Liberal Arts and Sciences, Osaka Prefecture University, 1-1 Gauken-cho, Naka-ku, Sakai, Osaka 599-8531, Japan
[3] Osaka Prefecture University, 1-4-22 Tsuzuyama-dai, Tondabayashi, Osaka 584-0086, Japan

* Corresponding author. Email: marian.saniewski@inhort.pl

Abstract
We have found that auxin, indole-3-acetic acid (IAA) substantially induces the formation of the secondary abscission zone in stem and petiole explants and in decapitated stem and petiole after excision of blade in intact plants of *Bryophyllum calycinum* when IAA at a concentration of 0.1% as lanolin paste was applied in the middle of these organs. The secondary abscission zone was formed at a few mm above of the treatment with IAA, and senescence of the part above abscission zone was observed. IAA additionally applied on the top of explants or top of the dacapitated stem or the debladed petiole totally prevented the secondary abscission zone formation and senescence induced by IAA applied in the middle of these organs. Possible mechanisms of the formation of the secondary abscission zone are discussed in terms of the interaction of auxin and ethylene.

Keywords
Bryophyllum calycinum; IAA; indole-3-acetic acid; petiole; senescence; stem

Introduction

Abscission is commonly associated with the sequence of processes resulting in shedding of multicellular organs including leaves, flowers, and fruits. In most cases, the site and the time of the formation of abscission zone is determined genetically in each organ. In certain plants, however, an additional abscission (the secondary abscission) zone has been known to be formed by some signals as possessing a specific functional competence between their neighboring cells, which is defined as transdifferentiation [1–3]. The secondary abscission has been observed primarily in vitro systems involving *Malus sylvestris* (L.) Mill. [4] and *Pyrus communis* L. pedicels [5], stems of *Impatiens sultani* Hook. [6–9], *Morus alba* L. [10] and *Citrus sinensis* L. [11], and *Phaseolus vulgaris* L. petiole explants [12,13]. The secondary abscission occurs in tissues away from a recognizable abscission zone in positions that are not defined in the intact plants. Most commonly secondary abscission occurs across the blade of a leaf, a pedicel or internode [9,14,15]. Specific protein determinants have been identified in leaf abscission cells that are preferentially expressed compared with neighboring (nonabscission) tissue [16–19]. This might be related to the mechanism of the secondary abscission zone formation.

Saniewski et al. [20] have reported that methyl jasmonate (0.5%) applied as a lanolin paste in different types of stem explants or in the debladed petiole induced the formation of the secondary abscission zone and senescence in *Bryophyllum calycinum*.

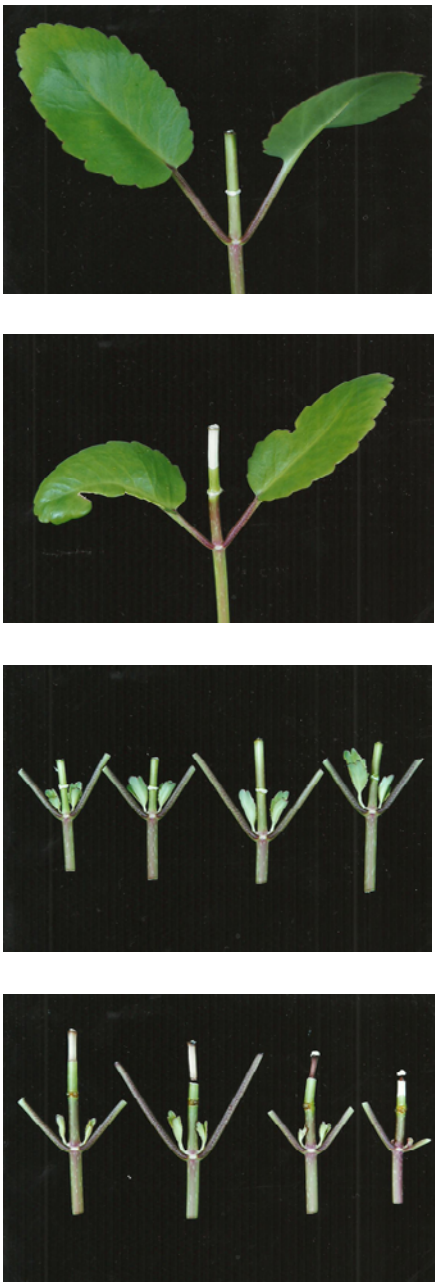

Fig. 1 The effect of IAA, applied in the middle of internode, on the formation of the secondary abscission in internode of *Bryophyllum calycinum* after decapitation of apical part of shoot. **a,c** Lanolin (control). **b,d** IAA treatment. Pictures were taken 12 (**a,b**) and 25 (**c,d**) days after treatment. To take photos, leaf blades with a part of petiole were excised.

On the other hand, the recent study reported that some of the external application experiments indicated that auxin can inhibit leaf senescence, whereas other experiments indicated that auxin can promote leaf senescence [21]. Auxin exogenously applied to plant tissues has also been well known to produce much higher ethylene, strongly affecting plant growth and development [22,23]. Accordingly, it is worth to study the dual physiological effects of auxin. We have found that auxin substantially induces the formation of the secondary abscission zone in stem and petiole explants and in decapitated stem and petiole after excision of blade of intact plants of *Bryophyllum calycinum* when only auxin was applied in the middle of these organs as a lanolin paste. In this paper we report detailed observations on the formation of the secondary abscission zone in several types of explants and decapitated stem of intact plants of *Bryophyllum calycinum*. Possible mechanisms of the formation of the secondary abscission zone are also discussed in terms of the interaction of auxin and ethylene.

Material and methods

One to eight-month-old plants of *Bryophyllum calycinum* Salisb. (Crassulaceae), propagated from epiphyllous buds arising in the marginal notches of the leaves, were used for the experiments. Different types of stem and petiole segments, decapitated stem, and debladed petioles of intact plants were used for treatment with IAA at a concentration of 0.1% in lanolin paste. Treatment with only lanolin was used as a control. Since the lengths of stem explants varied, ranging from almost 2.0 to 5.0 cm, each experiment was repeated 4 to 6 times with at least 10 explants or decapitated plants per treatment. Treatments with or without auxin were as follows.

Experiment 1

Naturally growing plants, a few months old, were decapitated below node and the top of decapitated internodes were smeared with lanolin only against dryness. Then, lanolin only or IAA 0.1% was applied in the middle of the internode as a ring. The treated plants were kept in natural light greenhouse conditions or in darkness. In some experiments, IAA 0.1% was applied in the middle of internode and additionally on the top of the decapitated internode and plants were kept in natural light greenhouse conditions (Fig. 1–Fig. 3).

Experiment 2

Internode segments (without node) excised from 1 to 4-month-old plants were treated in the middle of the segments with lanolin only (control) or with IAA 0.1% and then kept in natural or inverted position in chambers with water containing papers at the base of these segments (Fig. 4).

Experiment 3

In naturally growing plants, a few months old, leaf blade was excised and the remaining petiole was treated in the middle with lanolin only (control) or with IAA 0.1%, and the opposite leaf was intact. Lanolin was also applied at the place of removed leaf blade (Fig. 5).

Fig. 2 The effect of IAA, applied in the middle of internode, on the formation of the secondary abscission in the internode of *B. calycinum* after decapitation of apical part of shoot. Left – lanolin (control); right – IAA treatment. Explants were kept in darkness after treatment. Pictures were taken 10 days after treatment. To take photos, leaf blades with a part of petiole were also excised.

Fig. 3 The effect of simultaneous application of IAA on the top of internode after decapitation of apical part of shoot and in the middle of internode on the formation of the secondary abscission. Left – lanolin (control); middle – IAA applied in the middle of internode; right – IAA applied on the top and in the middle of internode. Pictures were taken 11 days after treatment.

Experiment 4

Petiole segments without leaf blade were used for the experiments. In the middle of the petiole segments lanolin only or IAA 0.1% was applied in the middle of petiole as a ring. Segments were kept in chamber with water containing papers at the base of segments (Fig. 6).

Results

After decapitation of apical part of shoot in naturally growing *Bryophyllum calycinum* morphological changes were not observed in the last internode but axillary buds were developed at base of leaves of the internode. When auxin at a concentration of 0.1% in lanolin paste was applied in the middle of the last internode, the secondary abscission was found at a few mm above the treatment. Chlorophyll disappeared and senescence was also observed in the part of stem above formed abscission zone. Axillary buds were also developed but a little smaller (Fig. 1). When the decapitated plants treated with IAA 0.1% in the same way as described above were kept in darkness, the formation of auxin-induced secondary abscission was found at a few mm above of the treatment. Chlorophyll disappearance was also observed (Fig. 2). When the last internode was treated with IAA 0.1% in the middle of the internode after decapitation of stem and additionally applied IAA 0.1% at the place of decapitation, the secondary abscission zone was not formed although it was normally induced in the middle of internode by the application of IAA. In this case, the disappearance of chlorophyll and senescence were not observed (Fig. 3).

In excised segments of internodes, without nodes, treated with IAA 0.1% in the middle of internode, the secondary abscission was induced at a few mm above of the treatment in acropetal direction. Chlorophyll disappearance and senescence were observed independent of orientation of explants, basal end down or basal end up (Fig. 4).

After excision of leaf blade, IAA 0.1% treatment in the middle of petiole inhibited petiole abscission and induced the secondary abscission at a few mm above of the treatment. The part above abscission zone finally died with disappearance of chlorophyll and anthocyanins (Fig. 5).

After excision of leaf blade, IAA 0.1% applied in the middle of the petiole induced the secondary abscission at a few mm above of the treatment. The part above abscission died and typical senescence symptoms were observed (Fig. 6).

It should be mentioned that different types of stem and petiole segments, decapitated stem, and debladed petioles of intact plants, showed absolutely positive reaction to induce the secondary abscission formation in each experiment after treatment with auxin.

Discussion

The formation of the secondary abscission zone has been defined as one of transdifferentiation processes. Phloem transdifferentiation from immature xylem cells has been well known, where *VASCULAR-RELATED NAC-DOMAIN6* (*VND6*) and *VND7* genes encoding NAM/ATF/CUC domain protein transcription factors act as key regulators of xylem vessel differentiation [1–3]. McManus et al. [12] have intensively studied the transdifferentiation of mature cortical cells to functional abscission cells in bean (*Phaseolus vulgaris*) plants. Abscission explants of bean were treated with ethylene to induce cell separation at the primary abscission zone. After several days of further incubation of the remaining petiole in endogenously produced ethylene, the distal two-thirds of the petiole became senescent, and the remaining (proximal) part stayed green. Secondary abscission zone is always formed at the junction between the senescing yellow and the enlarging green cells.

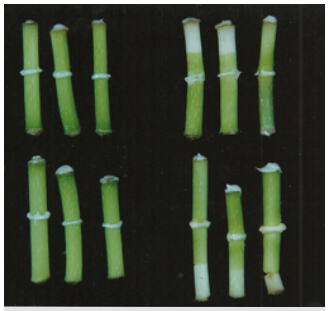

Fig. 4 The effect of IAA on the formation of the secondary abscission in segments of internode stem of *B. calycinum* after treatment in the middle of the internode. Upper row – natural position, lower row - inverted position; left – lanolin (control); right – IAA treatment. Segments were excised from 3-month-old plants and kept in natural and inverted position after treatment for 8 days.

Fig. 5 The effect of IAA on petiole abscission and the formation of the secondary abscission. IAA was applied in the middle of petiole after excision of leaf blade. The opposite petiole was treated with lanolin only (control).

Fig. 6 Formation of the secondary abscission zone in petiole segments after application of IAA in the middle of internode. Left – lanolin (control); right – IAA treatment.

We have already reported that methyl jasmonate induced the formation of the secondary abscission zone not only in explants but also in decapitated shoot of intact plants of *Bryophyllum calycinum* [20]. In this case endogenously or exogenously supplied auxin substantially inhibited secondary abscission zone. On the other hand, the formation of the secondary abscission zone has also been reported to be induced by applying auxin to the exposed primary abscission surface after the bean pulvinus (*P. vulgaris*) was shed, provided that ethylene was added [12]. Then, the orientation of development of green and yellow tissue was reversed; the distal tissue remained green and the proximal tissue yellowed. In this study, exogenously applied auxin has been clarified to induce the formation of the secondary abscission zone accompanied by senescent tissues as shown in Fig. 1–Fig. 4.

Mechanisms of the secondary abscission zone formation induced by exogenously applied auxin in *B. calycinum* have not been clear yet, but it might depend on the timing of exposure to ethylene induced by auxin and/or auxin gradient and its polar transport in tissues of *B. calycinum*. Wilson et al. [6–9] on the basis their studies on the formation of the secondary abscission in stem explants of *Impatiens sultani* proposed that the secondary abscission developed within a morphogenic field generated by the auxin gradient, although it was not determined how the auxin gradient could contribute to the differentiation processes.

Simone [13] continued elegant studies on the role of IAA and ethylene in bean petiole explants during IAA-induced secondary abscission zone formation. The secondary abscission forms at a site along the petiole which was removed from the primary zone and governed by the concentration of IAA added. During the formation of the secondary zone, the concentration of free IAA in the petiole tissue changed greatly. The fresh tissues produce the lowest amounts of ethylene and the senescent tissues at a secondary separation produce the most ethylene.

The function of ethylene derived from exogenously applied auxin in induction of the formation of secondary abscission zone might not be excluded. In *B. calycinum* irregular abscission zone develops in lower part of the internode after excision of the distal node and the shoot. Development can be accelerated by ethephon and inhibited by auxin [24]. McManus et al. [12] established that ethylene provides one of requirements for the formation of secondary abscission zone in the *Phaseolus vulgaris* explants excised from the primary leaves and that the auxin concentration can provide positional information for the formation of secondary abscission zone. The secondary abscission zone was formed precisely at the junction between the senescing yellow and the green tissues. McManus et al. [12] also showed that β-1,4-glucanhydrolase activity was induced in a secondary abscission zone (directed by endogenous ethylene) at the side of the yellow-green junction. Prior to the formation of the abscission zone in leaves of *Populus* trees, a new auxin maximum is established, which likely provides positional cues for the formation of the abscission zone. The analysis of microarray results revealed that several genes encoding auxin transporters were strongly downregulated during abscission, suggesting their involvement in the formation of the auxin maximum in the leaf axil [19]. Further studies on the mechanism of the formation of secondary abscission will be required in terms of auxin gradient and/or auxin polar transport including the expression of their related genes in *B. calycinum*.

References

1. Pang Y, Zhang J, Cao J, Yi SY, He XQ, Cui KM. Phloem transdifferentiation from immature xylem cells during bark regeneration after girdling in *Eucommia ulmoides* Oliv. J Exp Bot. 2008;59:1341–1351. http://dx.doi.org/10.1093/jxb/ern041
2. Yamaguchi M, Goué N, Igarashi H, Ohani M, Nakano Y, Mortimer JC, et al. VASCULAR-RELATED NAC-DOMAIN6 and VASCULAR-RELATED NAC-DOMAIN7 effectively induce transdifferentiation into xylem vessel elements under control of an induction system. Plant Physiol. 2010;153:906–914. http://dx.doi.org/10.1104/pp.110.154013
3. Reusche M, Thole K, Janz D, Truskina J, Rindfleisch S, Drübert C, et al. *Verticillium*

infection triggers VASCULAR-RELATED NAC-DOMAIN7-dependent de novo xylem formation and enhances drought tolerance in *Arabidopsis*. Plant Cell. 2012;24:3823–3837. http://dx.doi.org/10.1105/tpc.112.103374

4. Pierik RLM. Induction of secondary abscission in apple pedicels in vitro. Physiol Plant. 1977;39:271–274. http://dx.doi.org/10.1111/j.1399-3054.1977.tb01882.x
5. Pierik RLM. Hormonal regulation of secondary abscission in pear pedicels in vitro. Physiol Plant. 1980;48:5–8. http://dx.doi.org/10.1111/j.1399-3054.1980.tb03210.x
6. Warren Wilson PM, Warren Wilson J, Addicott FT, McKenzie RH. Induced abscission sites in internodal explants of *Impatiens sultani*: a new system for studying positional control. Ann Bot. 1986;57:511–530.
7. Warren Wilson J, Warren Wilson PM, Walker ES. Abscission sites in nodal explants of *Impatiens sultani*. Ann Bot. 1987;60:693–704.
8. Warren Wilson J, Walker ES, Warren Wilson PM. The role of basipetal auxin transport in the positional control of abscission sites induced in *Impatiens sultani* stem explants. Ann Bot. 1988;62:487–495.
9. Warren Wilson J, Palni LMS, Warren Wilson PM. Auxin concentration in nodes and internodes of *Impatiens sultani*. Ann Bot. 1999;83:285–292. http://dx.doi.org/10.1006/anbo.1998.0820
10. Suzuki T. Shoot-tip abscission and adventitious abscission of internodes in mulberry (*Morus alba*). Physiol Plant. 1991;82:483–489. http://dx.doi.org/10.1111/j.1399-3054.1991.tb02936.x
11. Plummer JA, Vine JH, Mullins MG. Regulation of stem abscission and callus growth in shoot explants of sweet orange [*Citrus sinensis* (L.) Osbeck]. Ann Bot. 1991;67:17–22.
12. McManus MT, Thompson DS, Merriman C, Lyne L, Osborne DJ. Transdifferentiation of mature cortical cells to functional abscission cells in bean. Plant Physiol. 1998;116:891–899. http://dx.doi.org/10.1104/pp.116.3.891
13. Simone F. Studies on abscission cell differentiation in *Sambucus nigra* and *Phaseolus vulgaris* [PhD thesis]. Palmerston North: Institute of Molecular Biosciences, Massey University; 2002.
14. Addicott FT. Abscission. Berkeley, CA: University of California Press; 1982.
15. Roberts JA, Whitelaw CA, Gonzalez-Carranza ZH, McManus MT. Cell separation processes in plants-models, mechanisms and manipulation. Ann Bot. 2000;86:223–235. http://dx.doi.org/10.1006/anbo.2000.1203
16. McManus MT, Osborne DJ. Evidence for the preferential expression of particular polypeptides in leaf abscission zones of the bean *Phaseolus vulgaris* L. J Plant Physiol. 1990;136:391–397. http://dx.doi.org/10.1016/S0176-1617(11)80025-8
17. McManus MT, Osborne DJ. Identification of polypeptides specific to rachis abscission zone cells of *Sambucus nigra*. Physiol Plant. 1990;79:471–478. http://dx.doi.org/10.1111/j.1399-3054.1990.tb02105.x
18. McManus MT, Osborne DJ. Identification and characterization of an ionically-bound cell wall glycoprotein expressed preferentially in the leaf rachis abscission zone of *Sambucus nigra* L. J Plant Physiol. 1991;138:63–67. http://dx.doi.org/10.1016/S0176-1617(11)80731-5
19. Jin X. The role of auxin in abscission of organs and tissues [PhD thesis]. Umeå: Department of Forest Genetics and Plant Physiology, Faculty of Forest Sciences, Swedish University of Agricultural Sciences; 2015.
20. Saniewski M, Ueda J, Miyamoto K. Methyl jasmonate induces the formation of secondary abscission zone in stem of *Bryophyllum calycinum* Salisb. Acta Physiol Plant. 2000;22:17–23. http://dx.doi.org/10.1007/s11738-000-0003-8
21. Hou K, Wu W, Gan SS. *SAUR36*, a SMALL AUXIN UP RNA gene, is involved in the promotion of leaf senescence in Arabidopsis. Plant Physiol. 2013;161:1002–1009. http://dx.doi.org/10.1104/pp.112.212787
22. Kan BG, Newcomb W, Burg SP. Mechanism of auxin-induced ethylene production. Plant Physiol. 1971;47:504–509. http://dx.doi.org/10.1104/pp.47.4.504
23. Hansen H, Grossmann K. Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. Plant Physiol. 2000;124:1437–1448. http://dx.doi.org/10.1104/pp.124.3.1437
24. Horton RF. The regulation of stem abscission in succulents. In: Pilet PE, editor. Abstract:

9th International Conference on Plant Growth Substances; 1976 Aug 30 – Sep 4; Lausanne, Switzerland. Lausanne: University of Lausanne; 1976. p. 152–153.

Auksyna efektywnie indukuje tworzenie się warstwy odcinającej u *Bryophyllum calycinum* Salisb. (Crassulaceae)

Streszczenie

W szeroko prowadzonych badaniach nad interakcją jasmonianów z auksyną w różnych procesach fizjologicznych wykazaliśmy, że auksyna IAA (kwas indolilo-3-octowy) indukuje tworzenie się wtórnej warstwy odcinającej w łodydze i ogonkach liściowych eksplantatów, w łodydze po dekapitacji i ogonkach liściowych po usunięciu blaszki liściowej naturalnie rosnących roślin *B. calycinum*, kiedy IAA w stężeniu 0.1% podano w paście lanolinowej pośrodku tych organów. W pracy szczegółowo omówiono możliwe mechanizmy tworzenia się wtórnej warstwy odcinającej indukowanej przez egzogennie podaną auksynę (w aspekcie interakcji auksyna–etylen) w różnego typu eksplantatach i po dekapitacji roślin *B. calycinum*.

