BIOLOGIA PLANTARUM (PRAHA)

12 (2) : 125—133, 1970

Growth Correlations in *Bryophyllum* Leaves and Exogenous Growth Regulators

R. DOSTÁL

Department of Botany, College of Agriculture, Brno*

Received June 3, 1969

Abstract. Growth correlations in leaves of *Bryophyllum* may be recognized by the development of marginal shoots varying in their particular lamina regions, the correlative inhibition increasing from the top to the base. Cytokinins extend their promoting action in *B. crenatum* leaves farther in the apical than in the basal direction. In the uppermost leaves of *B. daigremontianum* they evoke the development of marginal shoots with flowers in the apical and of vegetative shoots in the basal region. Less suitable for this research are auxins, gibberellins, and triiodobenzoic acid which are unable to stimulate the development of marginal shoots on leaves grown out under short days if not supplemented by cytokinins. Only TIBA induces under long days formation of adventitious shoots directly from the lamina surface on leaves developed below the terminal ring fasciation exhibiting at free ends of connate leaves normal marginal shoots. Under short days a complete reduction of lateral teeth takes place in *B. verticillatum* both on the extremity of ring fasciation and on the uppermost leaves, the marginal shoots being formed under the subsequent long days only in the angles between the ring fasciation parts. Furthermore the correlative inhibition of the leaf causes anisophylly in marginal shoots in the same way as that of axillaries in *Bryophyllum*.

In spite of numerous papers dealing with the development of marginal shoots in *Bryophyllum* species either on entire plants or on detached leaves, this material seems to be very little employed for establishing growth correlations in the leaves themselves. As these latter reveal some parallels to the correlations in stems demonstrated by surgical manipulations and elucidated later more profoundly with the aid of exogenous growth regulators, these compounds were utilized in the present paper whereas surgical methods had been largely used in the previous ones (DOSTÁL 1930, 1947, DOSTÁL and MAŠKOVÁ 1949, UHROVÁ 1935).

Material and Methods

Greenhouse specimens of *B. verticillatum*, *daigremontianum*, *rosei*, and *crenatum*, grown for many years in pots with garden earth under common

* *Address:* Brno, Zemědělská 1, Czechoslovakia.

growth conditions were treated by means of fine scalpels with IAA, NAA, MCPA, GA_3, BA, TIBA, and MH respectively in lanolin pastes to enable their localized application. Owing to the continuous culture the stems showed zones formed under long days alternating with those developed under short days. For instance, a stem of *B. verticillatum* exhibited on May 30, 1969 still three clear-cut leafy zones showing 16 whorls with marginal shoots followed by 12 whorls with no marginals and 17 new whorls with marginals. It is to be noted that the development of marginal meristems could be retained under long days by light of low intensity so that in addition finally no lateral teeth appeared at the lamina margins. It is well known that detached leaves develop marginal shoots under all illumination conditions, even in darkness, but the morphological experiments reported here were performed mostly on whole plants. Measuring or weighing of the experimental materials was here omitted in contrast to the former reports concerning above all the so-called mass relations. The results to be discussed are grouped according to the effectiveness of the growth substances used here, starting with cytokinin overcoming also the apical dominance in stems (SACHS and THIMANN 1964, 1967).

Results

Cytokinins

Leaves of intact *B. crenatum* stems show even under long days a complete growth inhibition of marginal meristems. Only rather rarely under certain circumstances a few apical leaf notches develop short shoots, the lamina middle and the base manifesting in all morphological experiments growth inhibitions. When the lamina base was treated with benzylaminopurine paste at its diplophyll-shaped folds, a strong marginal shoot development took place from the treated notches and in addition from the adjacent upper unsmeared ones, proving that the action of this cytokinin travels for some distance in the lamina acropetally. But the middle and the apical part of these leaves showed no trace of marginal shoots. Still more pronounced appeared this apically directed extension of the cytokinin effect from the base in leaves situated higher up on the stem with their quite flat lamina though the apical notches here also showed no growth of shoots. In other experiments only one of the lateral notches in the middle of the lamina margin was treated with the result that marginal shoot development was obtained not only in the treated notch, but also in the next apically oriented one, whereas the basally oriented notches were less or not promoted. The same result was obtained when a longitudinal incision was made close to single opposite notches, one of which was treated with BA paste (Fig. 1, a).

Similarly the action of the cytokinin paste applied to the lamina base did not move basipetally, that is, through the petiole into the corresponding axillary bud, as this latter remained regularly quite small, even when the lamina base developed very strong marginal shoots. On the contrary, an

Abbreviations used: NAA = α-naphthylacetic acid, MCPA = 4-methyl-2-chlorophenoxyacetic acid, GA_3 = gibberellic acid, BA = 6-benzylaminopurine, TIBA = 2, 3, 5-triiodobenzoic acid, MH = maleic hydrazide, B. = *Bryophyllum*.

acropetal transport of the cytokinin action could be frequently observed when only an axillary bud was treated therewith and developed as well as its serial bud very strongly; simultaneously also the marginal shoots on the non-treated subtending leaf developed at its base as evidence of the acropetal movement of the cytokinin effect through the petiole. Yet, in spite of a much smaller distance no cytokinin effect could be seen in these experiments in

Fig. 1. a) *Bryophyllum crenatum*. Leaf incised on both sides near one lateral notch one side of which was treated with benzylaminopurine (above) showing strong development of its marginal shoot and extending more apically than basally. The opposite lamina half remained narrower and exhibited only a very small marginal bud near the incision (the experiment lasted from December 29, 1968 to May 2, 1969).
b) *B. daigremontianum*. The uppermost leaf on the intact stem treated at the lateral notches (above) with BA, producing under short days at the apical half flowering marginal shoots and at the basal half vegetative shoots only. The opposite untreated leaf half lacks marginal shoots and dries out from the margin (September 8, 1968 to May 14, 1969).

the opposite untreated axillary bud which did not grow at all or only into a slight shoot soon losing its leaves and decaying.

In contrast to *B. crenatum*, the species *B. verticillatum* already develops marginal shoots under long days on young still vertically-directed leaves, whereas short-day leaves of intact plants show no trace of marginal shoots and of their carriers, only lateral teeth being then of course visible in decreasing number. This behaviour, ascribed by MEYER (1953), GÖTZ (1953), KÖRNER (1955), SIRONVAL (1958) and others to photoperiodism in spite of the opposite effect obtained in detached leaves seems to be more correctly explained by RESENDE (1959) as a growth correlation depending according to him on "auxin-antiauxin balance". In fact, a cytokinin paste applied in *B. verticillatum* to the apices of these short day leaves promoted the de-

velopment of their marginal shoots on intact plants, as they are released from the correlative inhibitions of growing stem, from its apical dominance, as is the case of axillary buds (SACHS and THIMANN 1964, 1967). Nevertheless, photoperiodism is very characteristic for *Bryophyllum* species with respect to their so-called long-short day "requirements" for flower formation (DOSTÁL 1950).

Because of its large leaves *B. daigremontianum* allows one to establish morphogenetic differences between the marginal shoots corresponding to those of the axillary shoots connected with the different region of the mother leaf or of the leafy stem respectively. In contrast to the known cases of marginal shoots flowering on plants deprived of the terminal and lateral inflorescences, intact plants were used in the present experiments and the leaves smeared with BA paste on the notches of one lamina side, but only at one side of the nodes. In spite of a strong flower formation at the top of the stem and on the upper laterals, the uppermost large leaves developed abundantly flowering shoots from the BA treated marginal notches, yet only at the apical part of the margin, whereas the more basal notches, though equally treated with BA, produced only vegetative shoots decreasing in size towards the lamina base (Fig. 1, b). The BA treated half of the lamina grew out more considerably than the opposite half with no treatment which wilted and later dried out. This corresponds to the promotion of leaf expansion by cytokinins (SCOTT and LIVERMANN 1956). The opposite leaves of the same nodes were at one lateral margin treated with GA_3 paste, but without effect on its marginal meristems, GA_3 travelling into the opposite leaves and enhancing there flower formation under short days as in other cases (ZEEVAART and LANG 1962). Accordingly, the uppermost leaves behaved in these experiments like intact stems with their nodes treated in leaf axils with BA. Lateral shoots presented the same differences in quality because the upper were flowering and the lower vegetative. Equally as the basal margin shoots were always vegetative, all the leaves on the lower stem nodes following the same BA treatment produced only vegetative shoots from all their notches. Apparently, the cytokinins do not change substantially the morphogenetic quality of shoots which they evoke from the marginal meristems. They can lower or even exclude only growth inhibitions owing to their attracting power vis-a-vis given "morphogenetic" material and their importance for research on growth correlations consists in this attraction.

Higher concentrations of benzylaminopurine applied in lanolin to the leaf notches provoked in all *B.* species a considerable swelling of the first internodes of marginal shoots with numerous additional ones which arose also from the leaf axils frequently disclosing certain symmetry relations in these shoots. But similar stronger pastes administered to the lamina surface apart from its margins did not in the present experiments induce initiation of such additional shoots even when the paste was included in the longitudinal incisions in the middle of lamina. The same is true of the smaller leaves with entire margins lacking lateral teeth and marginal meristems like leaves provoked by MH treatment. Such leaves occur normally at the base of marginal shoots in *B. verticillatum*, *daigremontianum*, and *rosei* and they represent bracts in the floral region, resulting probably from some morpho-

genetic inhibitions, like scales on winter buds possessing the same significance for releasing the inhibitions in the plant, enabling in this way formation of normal foliage leaves. Marginal shoots of *B. crenatum* agree in this respect with the axillaries of all four *Bryophyllum* species under study since they show marginal buds even on their lowest leaves.

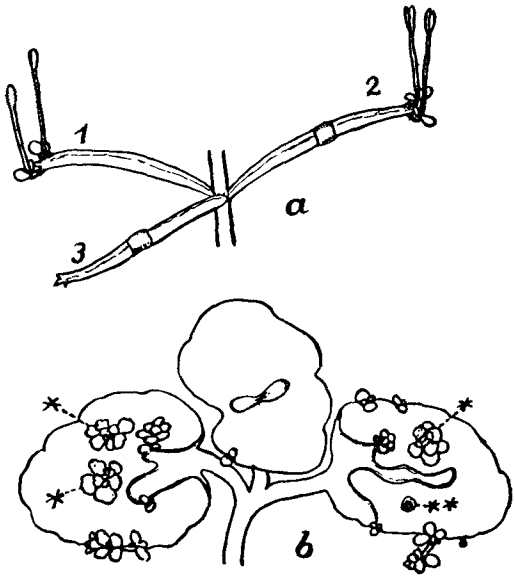

Fig. 2. a) *B. verticillatum*. One of the whorls with short day leaves 1 and 2 treated at the top with BA paste and besides leaves 2 and 3 with a ring of GA_3 paste, showing GA_3 effect only in the BA treated leaves (December 7, 1968 to February 22, 1969).
b) *B. crenatum*. The upper part of a stem treated with TIBA paste at the bottom showing leaves with adventitious shoots at the lamina surface (marked by asterisks; shoot fallen off-two asterisks) (August 28, 1968 to February 10, 1969).

Gibberellins

The easy movement of GA_3 in the plant in all directions could be observed also in *B. verticillatum* if treated with GA_3 paste either on the leaves or on the stem axis and grown under long days. The marginal shoots thereafter develop very long second and subsequent internodes and narrow leaves similarly above and below the place of GA_3 application. But leaves under short days lacking marginal shoots as well as their carriers showed no effect of GA_3. In no case a parallel to the GA_3 importance for the apical dominance in the stem, as shown in flax seedlings (DOSTÁL 1966), could be seen on fully grown leaves of *B. crenatum* even if detached. The original preponderance of the marginal shoots near the leaf apex in all *B.* species may be nevertheless due to substances transported from below (among others gibberellins) directly into the growing top of the stem as well as into the top of the still young leaves.

The necessity of combination of GA_3 with cytokinin to induce the growth of marginal shoots even on leaves developed under short day conditions, could be easily demonstrated on whorls of *B. verticillatum*. Two leaves of each whorl were treated at their top with BA paste and, besides, one of them received a ring of GA_3 paste in the middle of the petiole length. The third leaf not treated at all showed no marginal shoot development till the end of the experiment whereas the other two formed both quite similarly prolonged marginal shoots as a mark of GA_3 movement across the node, but effective only in the cytokinin treated leaves (Fig. 2, a).

Auxins

In accordance with the known inhibiting influence of auxins on buds, IAA or NAA pastes applied to the leaf tip or to lateral notches with meristems inactivated by short day conditions were for instance in *B. verticillatum* without effect. Detached leaves of *B. crenatum* developed marginal shoots normally at a certain distance from the IAA paste spot, but showed no prevalence of notches directed basally or apically. Similarly the treatment of detached leaves at the base or at the top showed no regularity in the marginal shoot development. The rather limited effectiveness of IAA in *Bryophyllum* leaves was also proved by MEYER (1953) and others.

The influence of IAA on the correlations between the axillaries and marginal shoots in *B. crenatum* was reported by the present author in a previous paper (DOSTÁL 1947). Similar experiments were repeated here on decapitated *B. crenatum* plants treated on the apical stump cut with pastes containing NAA or MCPA of relatively stronger concentrations. The growth of the upper axillaries was thereby retained and even the uppermost node was killed but its leaves remained alive and developed their marginal shoots normally, the toxic effect of these compounds being limited to the uppermost stem parts, from which the leaves though still adhering were physiologically detached. Among the similarly decapitated but untreated plants only the most vigorous showed some marginal shoots which were not retained by strongly outgrowing upper axillaries, these exceptions affording the evidence that a strong vigour in general does obscure growth correlations. Following a similar treatment of decapitated *B. crenatum* stems with IAA paste of lower inhibitory influence the axillary buds at the uppermost nodes grew out later and often differently, one of them forming a long shoot and the other a rosette-shaped axillary developed marginal shoots in contrast to the opposite leaf with marginal meristems retained by its long axillary. The inhibitory influence on marginal meristems depends, according to this, upon the morphogenetic nature of the inhibiting organs, all the other conditions being equal.

Triiodobenzoic Acid

TIBA paste administered to the marginal notches of leaves in *B. verticillatum*, *daigremontianum* or *rosei*, developed under short day, equally as IAA or GA_3 did not promote the growth of their meristems. It retains, however, not the growth of marginal shoots on leaves developing under favourable light conditions, and causes thereafter the well-known malformations which may suggest some morphogenetic interpretations. Of some interest seemed to be in this respect the upper leaves of *B. crenatum* stems after these latter had been treated near the base with TIBA paste and left to grow under long days. They were finally terminated with a ring fasciation preceded by one pair of leaves rather triangular than oval in shape, with an uneven lamina surface due to proliferations of mesophyll cells between the veins from which adventitious shoot arose apart from margins and diplophyllous excrescenses (Fig. 2, b). As noted above, cytokinin pastes did not evoke such adventitious formations on places originally lacking meristems.

In the same way the TIBA-treated *B. verticillatum* stem very often ex-

hibited in its lower part a following sequence of whorls if grown under long days: the lowest TIBA-influenced whorl had the first leaf pair on marginal shoots free and only the second connate to a ring fasciation. On the subsequent whorl such a ring fasciation was already formed by the first leaf pair of marginal shoots. The following 3 to 4 whorls showed no ring fasciation, but one of them produced in addition 2 to 5 adventitious shoots at a distance

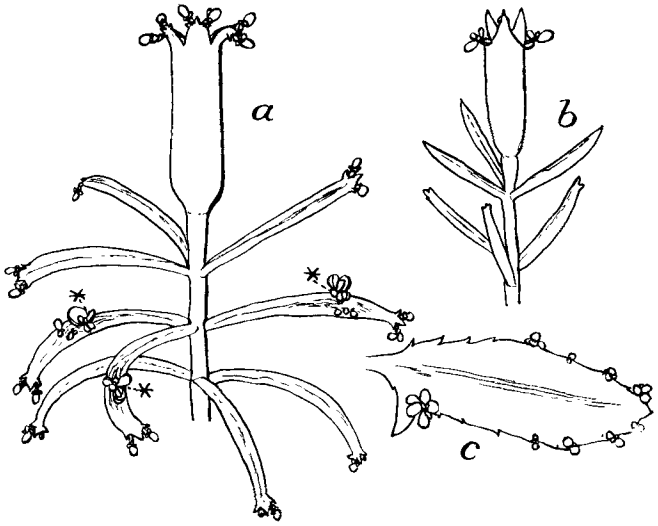

Fig. 3. a) *B. verticillatum.* TIBA-treated stem grown under long days showing adventitious buds (marked by asterisks) on one of the upper whorls and marginal shoots at the end of free parts of the terminal ring fasciation (July 24, 1968 to February 21, 1969).
b) *B. verticillatum,* grown under short days showing a complete reduction of lateral teeth on the upper leaf whorl and on the free parts of the ring fasciation which at the shift to the long days produced normal single marginal shoots in the angle between its free parts (January 25, 1969 to May 5, 1969).
c) *B. rosei.* Leaf with a large tooth at its base (below) with a large marginal shoot above it (May 17, 1969).

of 6 to 12 mm from the top with normal marginal shoots. These adventitious structures emerged from longitudinal ridges and exhibited no such regularity in their phyllotaxy as do normal marginal shoots of this species. The ring fasciation at the top of the stem was terminated by normal marginal shoots (Fig. 3, a). On the contrary, plants treated equally with TIBA but grown under short days developed no marginal shoots on leaves showing regularly a successive decrease in the number of lateral teeth on the leaves so that the uppermost free leaves were terminated with one point only. Similarly the upper non-connate parts of the subsequent ring fasciation ended with one point only with no trace of teeth and marginal meristems, but under subsequent long days normal single marginal shoots appeared on this ring fasciation in the angle between the free parts (Fig. 3, b). Such a displacement of these otherwise quite typical marginal shoots supports the above mentioned growth correlation, that only notches between the lateral teeth may conserve the meristem remainder originating from the early embryonic stage of leaves.

Leaves incised in their embryonic stage behaved similarly, frequently showing more notches and therefore more marginal shoots on the new artificial margins. Leaves of *B. rosei* often possess on one side of their base large natural incisions developing much larger shoots than do other notches near small teeth (Fig. 3, c).

Finally TIBA may render more conspicuous growth correlations between the leaves and the lateral structures without regard to whether they are axillary or marginal shoots. TIBA paste administered to *B. verticillatum* very often resulted in that out of the first two seemingly opposite leaves only the consistently larger leaf opposite the mother lamina (ablaminal) formed ring fasciation whereas the other (adlaminal) one remained flat as seen in the ouverture of this small asymmetric ring fasciation. Thus, the two first leaves of the marginal shoots are really not precisely opposite, corresponding in this respect to the second leaf pair of the axillaries in *Bryophyllum* species the adaxial leaf of which opposite to the subtending leaf being also larger and inserted lower on the axillary shoots, as is the ablaminal leaf of the marginal shoots. According to this identical behaviour of both these lateral structures, the subtending or mother leaf respectively exerts a primary correlative influence with respect to the induction of anisophylly both in axillary and marginal shoots.

References

DOSTÁL, R.: Versuche über Massenproportionalität bei der Regeneration von *Bryophyllum crenatum*. — Flora N. F. **24** : 240—300, 1930.

DOSTÁL, R.: On growth correlation in elongating stem region of *Bryophyllum crenatum*. — Věstník Kr. čes. sp. n. n. **13** : 1—19, 1947.

DOSTÁL, R.: Morphologische Experimente mit *Bryophyllum verticillatum*. — Acta Acad. sci. nat. Morav.-Silez. **22** : 57—98, 1950.

DOSTÁL, R.: Growth correlations in decapitated *Linum*-seedlings as possible test for kinin and gibberellin. — Flora A, **156** : 498—509, 1966.

DOSTÁL, R., MAŠKOVÁ, J.: On the morphogenesis of the buds on leaves of *Bryophyllum crenatum*. — Acta Acad. sci. nat. Morav.-Silez. **21** : 1—39, 1949.

GÖTZ, O.: Über Brutknospenentwicklung der Gattung Bryophyllum im Lang- und Kurztag. — Z. Bot. **41** : 445—482, 1953.

KRÖNER, E.: Experimentelle Beiträge zum Photoperiodismus der vegetativen Vermehrung der Gattung Kalanchoë. — Flora **142** : 400—465, 1955.

MEYER, G.: Über photoperiodische Beeinflussbarkeit der Brutknospenbildung bei *Bryophyllum crenatum*. — Z. Bot. **41** : 247—256, 1953.

MOTHES, K.: Die Bedeutung der Kinetinforschung zum Verständnis der pflanzlichen Korrelationen. — Ber. deutsch. bot. Ges. **74** : 24—42, 1961.

RESENDE, F.: Über die Brutknospen-Entwicklung bei Bryophyllum und ihre Vererbung. — Ber. deutsch. bot. Ges. **72** : 3—10, 1959.

SACHS, T., THIMANN, K. V.: Release of apical buds from apical dominance. — Nature **201** : 939—940, 1964.

SACHS, T., THIMANN, K. V.: The role of auxins and cytokinins in the release of buds from dominance. — Amer. J. Bot. **54** : 136—144, 1967.

SCOTT, R. A. J., LIVERMANN, J. L.: Promotion of leaf expansion by kinetin and benzylaminopurine. — Plant Physiol. **31** : 321—322, 1956.

SIRONVAL, C.: Action of day length upon the formation of adventitious buds in *Bryophyllum tubiflorum*. — Nature **182** : 1171, 1958.

UHROVÁ, A.: Über die Wechselbeziehungen der Knospen bei *Bryophyllum crenatum*. — Flora N.F. **29** : 260—286, 1935.

ZEEVAART, J. A. D., LANG, A.: The relationships between gibberellin and floral stimulus in *Bryophyllum daigremontianum*. — Planta **58** : 531—542, 1962.

R. DOSTÁL, Vysoká škola zemědělská, Brno: **Korelace růstu v listech *Bryophylla* a exogenní růstové regulátory.** — Biol. Plant. **12** : 125—133, 1970.

Korelace růstu v listech *Bryophylla* se mohou poznat z vývinu marginálních prýtů, odchylného v jejich jednotlivých krajinách, jak se korelační inhibice zesilují od vrcholku k basi. Účinek cytokininů se v listech *B. crenatum* šíří z místa aplikace ve směru apikálním dále než basálním. V nejhořejších listech *B. daigremontianum* cytokininy vyvolávají v jejich apikální krajině vývoj marginálních prýtů s květy a v basální krajině vývoj vegetativních prýtů. Méně se pro tyto pokusy hodí auxiny, gibereliny a trijodbenzoová kyselina, neschopné stimulovat vývin marginálních prýtů na listech vyrostlých v krátkých dnech, nejsou-li doplněny cytokininy. Jen TIBA zavádí za dlouhých dnů tvorbu adventivních prýtů přímo z povrchu čepele, a to na listech pod terminální ascidií, jevící na volných koncích srostlých listů typické marginální prýty. V krátkých dnech dochází u *B. verticillatum* k úplnému potlačení postraních zubů jak na konci ascidie, tak i na nejhořejších listech, takže se v následujících dlouhých dnech marginální prýty tvoří jen v úhlech mezi volnými částmi ascidie. Mimoto korelační inhibice listu působí anisofylii marginálních prýtů stejně jako anisofylii prýtů úžlabních u *Bryophylla*.