INFLUENCE OF GROWTH REGULATORS (IBA, BA) ON ANATOMICAL AND MORPHOLOGICAL CHANGES IN BROMELIADS IN IN VITRO CULTURE

RENATA GALEK¹, KRYSZYNA KUKULCZANKA²

¹University of Agriculture,
Cybulskiego 34, 50-205 Wrocław, Poland

²Botanical Garden, University of Wrocław,
50-335 Wrocław, Poland

(Received: November 23, 1995. Accepted: July 3, 1996)

ABSTRACT

The subject of study were *Tillandsia coronata* and *Guzmania monostachya*. The material has been obtained by means of in vitro propagation. The plants were grown for 18 weeks on various kinds of media. Morphological changes were recorded in both species subjected to action of growth regulators. The changes in plant habit were linked with anatomic build. The effect of cytokinin BA upon growth of the stem pith was found, transversely to its axis, through development of numerous meristematic centres and growth and development of adventitious shoots. Leaves of plant grown on media containing cytokinin BA were build of a higher number of cell layers of assimilation parenchyma. In plants grown on media with addition of cytokinin the size of stomatal cells was smaller and was accompanied by analogous changes in size of epidermis cells proper. The bushy type of the plants, caused by presence of cytokinin in medium, resulted from the increase of thickness and breadth of leaves and growth of the stem pith, with simultaneous inhibition of cells' elongation. Auxin IBA did not favour the growth of the existing axillary shoots, but stimulated elongation of the stem pith. The stomata of plants of both species grown on media with addition of auxin were bigger. As result of the applied growth regulators a higher frequency of appearance of binucleate cells was found in parenchyma cells of the stem and leaves in both the species studied.

KEY WORDS: growth regulators, IBA, BA, anatomy, *Bromeliaceae*, in vitro, RM medium.

INTRODUCTION

The methods of vegetative propagation are preferred by researchers as allowing to maintain the plant's genotype (de Bruyn and Ferreira, 1992). However, plants obtained by propagation in vitro may differ in appearance from those the explants were taken, particularly under the influence of the growth regulators used. Mekers (1977), Pierik (1987), Kukulczanka et al., (1989, 1994) observed morphological changes in *Tillandsia coronata* and *Guzmania monostachya*, using growth regulators in clonal propagation in in vitro conditions.

The observed changes in plant habit, direction of organogenesis, growth and development of plants take their source from anatomical changes originating in result of differentiated cell reactions to the growth regulators used (Sorokin and Thimann, 1964, cit. after Malcolm and Wilkins, 1969, Wareing and Philips, 1970). The action of synthetic auxins upon tissue cultures is varied. Some of them stimulate cell division or cell growth according to the kind of tissue they are influencing (Steward and Shantz, 1956; cit. after Malcolm and Wilkins, 1969). Exogenous cytokinins induce mitotic divisions, cause the release of axillary buds from under the domination of the apical meristem and their further growth and development; they may also be the reason of formation of new meristematic centres and differentiation of ad-

ventitious shoots from them, accompanied by differentiation of vascular tissue (Bergman, 1964; cit after Malcolm and Wilkins, 1969).

The analysis of changes in anatomical build occurring under the influence of gibberellic acid (GA3), abscisic acid (ABA) and cytokinin was carried out by Buchheim and Burkhardt (1994) in *Paeonia lactiflora*. Investigations confirmed the significant part of cytokinin in two processes: firstly, in inhibiting growth and development of the apical meristem, and secondly, in formation of new adventitious meristems from subepidermal parenchymatic tissue.

In connection with occurrence of morphological changes in bromeliads propagated in in vitro conditions (Kukulczanka et al., 1987, 1994), using exogenous auxin and cytokinin, it was useful to investigate the anatomical changes in these plants, for it may be supposed that anatomical changes are the reason of the earlier observed differences in morphology.

MATERIAL AND METHODS

The subject of study were leafy seedlings of *Tillandsia coronata* and *Guzmania monostachya*. The plants were grown in vitro through eighteen weeks on liquid RM medium or agar RM medium (Reinert and Mohr, 1967) including growth

regulators, i.e., 0.5 mg/dcm³ IBA or 0.5 mg/dcm³ BA. For anatomical analysis selected were the most characteristic plants, in fours for each of the given combinations.

The plant material was fixed in acet-alkohol, leaves and stem piths separately. Next the plant parts were dehydrated and carried through a sequence of alcohol solutions (ethyl alcohol and butyl alcohol). The material has been embedded in paraffin and cut by means of a microtome into 8-10 µm thick sections. The preparations were dyed with astra blue and basic fuchsin and carried through sequences of xylene- and alcohol solutions, according to the method of Teleżyńska and Teleżyński (1973).

Observations of longitudinal sections of the stem pith were carried out, considering the formation of lateral and adventitious meristems. Analysed were the transversal and longitudinal sections of leaves (12 in each combination) by determination of the size of stomatal cells (measurements were performed on 12 sections, measuring 30 cells on each of them), and the number of xylem-phloem bundles on transversal sections of the leaf. Determined was also the number of cells between conducting bundles and the distances between them, as well as breadth and thickness of the leaves, and the frequency of binucleate cells in stem pith (four repetitions – 120 cells in each) and in the parenchyma tissue of the leaf.

Analysed were four plants from every combination by taking three leaves from each plant. The most characteristic preparations were used for producing drawings and photographs.

RESULTS

Microscopic observations revealed changes in anatomic build of shoots and leaves in bromeliads in effect of the growth regulators used.

Fig. 1. Longitudinal section of lateral meristem of *Guzmania monostachya* on agar RM medium (100 x).

Shoot: differences in number, growth and development of shoots

Tillandsia coronata and *Guzmania monostachya* have shortened shoots and their leaves are arranged in a rosette. The phylotactic pattern of the stem is spiral. The presence of axillary or adventitious shoots has not been found macroscopically in plants grown on control media or those including auxin, whereas they occurred in great number in plants grown on media containing cytokinin.

Microscopic observations of both species, grown on control media for 18 weeks, revealed the occurrence of 1 to 2 underdeveloped axillary shoots with tiny leaf primordia in leaf axils growing at the shoot base. (Fig. 1).

Auxin IBA did not favour the growth and development of lateral meristems, but stimulated growth and development of the apical meristem (Fig. 2b, e). Therefore the plants were bigger and had more leaves.

Cytokinin BA inhibited growth and development of the apical meristem, but stimulated growth and development of lateral meristems and also the formation of numerous adventitious meristematic centres. New shoots approximated in size the basal shoot and were characterized by a good leafage (Fig. 3). The number of shoots was also affected by consistency of the medium. There were more of them on liquid medium, i.e., 3-7 (Fig. 2c), and 2-5 on agar medium (Fig. 2f).

Fig. 2. Schemes of longitudinal sections of the stem in *Tillandsia coronata*.

Leaf: differences in length of stomatal cells, number of conducting bundles, thickness and breadth of leaf

The leaves of both plants are of similar build, but in *Tillandsia coronata* there are more cell layers and they are therefore thicker than the leaves of *Guzmania monostachya*. The cells of epidermis proper have sinuous anticlinal cell walls. In bromeliads the guard cells are arranged in rows separated by cell layers of epidermis proper. Cells forming the stoma are surrounded by 4-5 subsidiary cells (Fig. 4). Subsidiary cells are smaller than epidermal cells proper. Under the layer of upper epidermis cells there were large, oval cells without chloroplasts. Below there were several cell layers forming the assimilation parenchyma. Between the abaxial epidermis and assimilation parenchyma there occur 2-3 cell layers with undulated cell walls. They are thin-walled cells accumulating water. The undulation of their walls was caused by dehydration of the cell during making the preparations. On both sides of the leaf there are peltate trichomes. The leaf blade has a parallel venation, characteristic of the monocotyledones.

Fig. 3. Longitudinal section of adventitious meristem of *Guzmania monostachya* on liquid RM medium with addition of cytokinin Ba.

- a) Differences in length of stomatal cells were found, caused by application of growth regulators (min value 22 μm , max value 41 μm) regardless of medium type. Auxin IBA caused an increase of stomata size by 4-6 % as compared with the control. Instead, cytokinin BA decidedly decreased their size. The smallest length of stomatal cells occurred in the epidermis of plants grown on agar RM medium supplemented with cytokinin. They were smaller than stomata of control plants, on average by 20% (Table 1a). These changes were accompanied by analogous differences in size of epidermis cells proper (Fig. 4).
- b) Differences in number of conducting bundles in leaves on liquid RM and agar RM medium were small in *Tillandsia coronata* (min 10, max 19), contrary to *Guzmania monostachya* (min 7, max 19). On liquid medium an increasing tendency in number of conducting bundles has been observed in both species in presence of growth regulators IBA and BA, on average from 20 to 110% as compared with the control. Instead, in plants grown on agar RM medium there occurred a distinct increase in number of conducting bundles in leaves, on average by 50%, as effect of acting of exogenous cytokinin (Table 1b).
- c) In both species the number of parenchyma cells occurring between conducting bundles was decidedly the highest (on average by 40-60% as compared with the control) on RM medium containing cytokinin BA. Auxin caused an increase in cell number by 30-45% on liquid medium, but not on agar medium (Table 1c). The smallest number of cells occurred in *Tillandsia coronata* (59) and the highest in *Guzmania monostachya* (194), regardless of kind of medium.
- d) Under the influence of growth regulators the distances between conducting bundles on transversal section of the leaf were usually shorter than in control plants (Table 1d). The lowest and highest values of this trait occurred in *Guzmania monostachya* (95-357 μm), while in *Tillandsia coronata* they fluctuated within the range 134-294 μm .

On agar RM medium the number of cells between conducting bundles and the distances between them were correlated with size of parenchyma cells. Under the influence of auxin there were less parenchyma cells and the distances between bundles were somewhat shorter than in control leaves. The number of cells between conducting bundles increased decidedly under the influence of cytokinin, but the distances between bundles were shorter than in control plants (except of leaves of plants grown on agar medium containing cytokinin). In this case the parenchyma cells were of lowest size.

As effect of a decrease in size of parenchyma cells, on liquid medium an increase in cell number has been recorded in case of both growth regulators, with a simultaneous decrease in distance between bundles.

TABLE 1. Influence of growth regulators and medium consistency on anatomical changes in bromeliad leaves.

Species \ Type of medium	Liquid RM medium			Agar RM medium		
	RM	RM +0.5 IBA	RM +0.5 BA	RM	RM +0.5 IBA	RM +0.5 BA
a. Length of stomatal cells (μm)						
<i>Tillandsia</i>	32	34	31	35	38	28
<i>Guzmania</i>	31	33	26	29	31	24
b. Number of xylem-phloem bundles						
<i>Tillandsia</i>	11	15	13	12	11	18
<i>Guzmania</i>	7	15	13	12	10	18
c. Number of cells between xylem-phloem bundles						
<i>Tillandsia</i>	65	95	74	84	60	118
<i>Guzmania</i>	100	130	152	98	73	161
d. Distances between xylem-phloem bundles (μm)						
<i>Tillandsia</i>	199	178	147	203	166	198
<i>Guzmania</i>	253	183	218	138	127	153
e. Breadth of leaf (μm)						
<i>Tillandsia</i>	2087	2775	1976	2649	1894	3707
<i>Guzmania</i>	1887	2930	3056	1798	1406	2900
f. Thickness of leaf (μm)						
<i>Tillandsia</i>	407	377	474	363	338	518
<i>Guzmania</i>	178	200	200	178	170	185
g. Percentage of binucleate cells in parenchymal tissue of the leaf						
<i>Tillandsia</i>	1,2	2,5	4,2	1,2	3,3	5,4
<i>Guzmania</i>	0,8	4,2	4,6	0,8	2,9	5,4
h. Percentage of binucleate cells in parenchymal tissue of the stem						
<i>Tillandsia</i>	5,4	6,7	10,4	4,6	9,6	9,6
<i>Guzmania</i>	4,6	9,6	6,3	4,6	8,8	9,6

- e) Under the influence of IBA and BA included in liquid RM medium an increasing tendency in leaf breadth in the bromeliad has been observed, on average by 30-55% in relation to the control, but also in agar RM medium with addition of cytokinin (on average by 40-60%). Instead, bromeliads leaves on agar RM medium with addition of auxin were distinctly narrower, on average by 25%, than in the remaining combinations (Table 1e). The lowest

Fig. 4. Surfacial sections of epidermis of *Guzmania monostachya*.

- values in this trait occurred in *Guzmania monostachya* (160 μm), and the highest in *Tillandsia coronata* (651 μm).
- f) Leaves of *Tillandsia coronata* were distinctly thicker than of *Guzmania monostachya*. Changes in leaf thickness effected by growth regulators were more distinct in *Tillandsia coronata* (Fig. 9) than in *Guzmania monostachya* (Table 1f), regardless of medium consistency. Auxin IBA caused a slight decrease in leaf thickness in both species, as compared with control (except of *Guzmania monostachya* on liquid medium). Cytokinin influenced the increase of leaf thickness on average by 10-30% (Figs 5c, f).
- g) In both species growing on control media binucleate cells occurred with a frequency of 4.6-5.4% (in stem) and 0.8-1.2% (in leaves). In case of stem pith cells growth regulators IBA and BA caused a double increase in frequency of

Fig. 5. Transversal sections of leaf fragments of *Tillandsia coronata*.

appearance of binucleate cells in both kinds of media. In parenchyma tissue of the leaf cytokinin BA influenced, more distinct than auxin IBA, the frequency of appearance of binucleate cells. They appeared in a 4-6 time higher number on liquid medium, and on agar medium in a 5-7 times higher number in relation to the control.

In result of the carried out observations changes in outlook of cell nucleim were found in *Tillandsia coronata* (Figs 7, 8), but not in *Guzmania monostachya* (Fig. 6). These changes may have originated during fixation of the objects.

Fig. 6. Binucleate cell – longitudinal section of longitudinal axis of shoot of *Guzmania monostachya* on agar RM medium with addition of cytokinin BA.

Fig. 7. Injured cell nuclei – transversal section of leaf of *Tillandsia coronata* on agar RM medium.

DISCUSSION

In the present study subject to analysis was the influence of growth regulators, i.e., auxin IBA and cytokinin BA, and also the influence of medium consistency on anatomo-morphological changes in bromeliads. Considered was also the occurrence of binucleate cells in assimilation parenchyma tissue of leaves and in parenchyma cells of stem pith.

On the grounds of microscopic observations of the stem pith a differentiation of the course of development of lateral and adventitious meristems, according to the used growth regulators, was recorded.

Fig. 8. Injured cell nuclei – transversal section of leaf of *Tillandsia coronata* on agar RM medium with addition of auxin IBA.

Auxin IBA inhibited the development of axillary buds in leaf axils at the shoot base. These were at the stage of meristem formation, with tiny leaf primordia. Their presence has not been recorded by Kukułczanka and Poturafa (1994). Instead, cytokinin inhibited the growth of the basal meristem and stimulated the development and growth of axillary buds and the formation of meristematic centres from which numerous adventitious shoots were originating. The participation of cytokinin BA in formation of new meristem centres and in differentiation of adventitious shoots have been observed by Bruyn and Ferreira (1992) during micropropagation of *Amaryllis belladonna* from twin scales, and by Buchheim and Burkhartl (1994) during induction of origin of adventitious meristems in *Paeonia lactiflora* embryos. The carried out observations have shown that cytokinin effects the development of xylem-phloem bundles. This is confirmed by studies carried out in 1964 by Sorokin and Thiman (cit. after Malcolm and Wilkins, 1969), which proved that the release of lateral meristems from under the inhibiting influence of the apical meristem takes place simultaneously with the differentiation of xylem and phloem elements. According to Bergmen (1964; cit. after Malcolm and Wilkins, 1969) this is connected with the influence of cytokinin on biosynthesis of lignin. The stabilization of vascular connections allows the further growth and development caused by a better supply with water and compounds diluted in it. Therefore, in order to attain a more effective utilization of regeneration in *in vitro* cultures in the first phase of propagation preferred is the organogenesis determined by cytokinins, which ensures a maximal number of new shoots. This is of significance in plants the vegetative reproduction of which in field or garden conditions is slow, e.g., in *Crinum macovani* (Slabbert and Bruyn, 1993), *Amaryllis belladonna* (Bruyn and Ferreira, 1992), *Paeonia lactiflora* (Buchheim and Burkhartl, 1994). After measurements of stomata and observations of cells proper of the epidermis the influence of auxin IBA on elongation of cells has been ascertained. The cells were bigger in comparison with the control. Instead, on cultures containing cytokinin BA stomata and the epidermis cells proper were smaller. Previous studies (Kukułczanka and Poturafa, 1994) have shown that after applying auxin IBA the leaves of *Tillandsia coronata*, and particularly *Guzmania monostachya* were longer in relation to the control or in comparison with leaves of plants grown on media with addition of cytokinin BA.

Fig. 9. Transversal sections of leaf fragments of *Tillandsia coronata* on: a) agar RM medium, b) with addition of auxin, c) with addition of cytokinin.

The study has shown that cytokinin stimulates cell division in vertical direction to the leaf axis, which manifested itself in an increase of its thickness and breadth of the leaf. It also causes an increase in stem diameter through activation of axillary buds and adventitious shoots. Intense divisions in transverse, but not longitudinal direction to the leaf axis and the growth of the stem pith caused a characteristic, unnatural bushy type of the plants. This has been observed earlier in the study by Kukułczanka and Czastka (1989), Kukułczanka and Poturafa (1994). However, after planting out the plants into *in vivo* conditions no constant growth anomalies were recorded.

The occurrence of binucleate cells in assimilation parenchyma of leaves and parenchyma cells of the stem in plants grown on control media is a characteristic phenomenon for both species and may evidence polysomy. However, the carried out measurements point to a significant impact of cytokinin on the occurrence of such cells. Accelerating cell division the cytokinins produce sometimes function disturbances in the karyokinetic spindle and heighten disturbances in course of cytokinesis (Pierik, 1987). In case of bromeliads photocytometric measurements should be carried out to determine the possible changes in the degree of cell ploidity.

CONCLUSIONS

The influence of the applied exogenous growth regulators on changes in anatomic build of bromeliads (*Tillandsia coronata* and *Guzmania monostachya*) propagated in in vitro conditions has been ascertained.

1. Auxin IBA causes elongation growth of cells in both species, which results in their larger dimensions, but inhibits the development of axillary shoots and the formation of new meristematic centres.
2. Cytokinin BA inhibits the elongation of cells and induces cell division in vertical direction to leaf axis. Cytokinin inhibits also growth and development of the basal meristem and stimulates the formation and further growth of adventitious and lateral meristems with a simultaneous differentiation of vascular tissue.
3. The result of cytokinin action is a bushy type of the plants. The plants are characterized by broad, thick and short leaves.
4. The liquid consistency of RM medium, particularly with addition of cytokinin BA, favourably affects the formation of meristematic centres and their further differentiation.
5. Growth regulators – auxin IBA and cytokinin BA – influence the increase in frequency of appearance of binucleate cells, particularly in the assimilation parenchyma of leaves.

ACKNOWLEDGEMENTS

We are most grateful to Alicja Błaszczuk and Doc. Dr. hab. Jan Cebart for their useful comments and suggestions, which greatly improved the manuscript.

LITERATURE CITED

- BUCHHEIM J., BURKHART L., 1994. Effect of exogenous gibberellic acid, abscisic acid, and benzyloadenine on epicotyl dormancy of cultured herbaceous peony embryos. *Plant Cell, Tissue and Organ Culture* 36: 1, 35-43: 35 ref.
- DE BRUYN H. H., FERREIRA D., 1992. In vitro propagation of *Amaryllis belladonna*. *Plant Cell, Tissue and Organ Culture* 31: 3, 179-184, 16 ref.
- KUKULCZANKA K., CZĄSTKA B., 1989. Propagation of some species of the *Bromeliaceae* family cultured in vitro. *Act. Hortic.* 251: 167-172
- KUKULCZANKA K., POTURAŁA D., 1994. Fitohormonalna regulacja bromelii (*Bromeliaceae*) w warunkach in vitro. *Prace Ogródu Botanicznego PAN, Powsin* 5/6: 133-138
- MALCOLM B. WILKINS, 1969. The physiology of plant growth and development. Mc Graw-Hill Publishing Company Limited
- MEKERS O., 1997. In vitro propagation of some *Tillandsioideaceae* (*Bromeliaceae*). *Act. Hortic.* 78: 311-320
- MEKERS O., VAN ONSEM J.G., 1983. In vitro propagation of *Vriesea* cultivar in comparison with ornamental *Bromeliaceae*. *Act. Hortic.* 131: 125-130
- PIERIK R. L. M., 1987. In vitro culture of higher plants. Martinus Nijhoff publisher, Dordrecht
- REINERT R. A., MOHR H. C., 1967. Propagation of *Cattleya* by tissue culture of lateral bud meristems. *Proc. American. Sci.* 91: 664-671
- SLABBERT M. M., DE BRUYN H. H., 1993. Regeneration of bulblets from twin scales of *Crinum macovani* in vitro. *Plant Cell, Tissue and Organ Culture* 33: 133-141
- TELEŻYŃSKA J., TELEŻYŃSKI H., 1973. Double fertilization in *Helianthus*. *Acta Soc. Bot.* 42: 323-343
- WAREING P. F., PHILIPS D. J., 1970. The control of growth and differentiation in plants. Pergamon Press Ltd.

WPŁYW REGULATORÓW WZROSTU (IBA, BA)
NA ZMIANY ANATOMICZNO-MORFOLOGICZNE U BROMELII W KULTURZE IN VITRO

STRESZCZENIE

Przedmiotem badań były *Tillandsia coronata* i *Guzmania monostachya*. Materiał uzyskano na drodze rozmnażania in vitro. Rośliny hodowano przez okres 18 tygodni na różnych rodzajach pożywek. Stwierdzono zmiany morfologiczne u roślin obu badanych gatunków poddanych działaniu regulatorów wzrostu. Zmiany pokroju roślin miały związek z budową anatomiczną. Stwierdzono wpływ cytokiny BA na rozrost rdzenia łodygi w kierunku poprzecznym do jej osi poprzez rozwój licznych centrów merystematycznych oraz wzrost i rozwój pędów przybyszowych. Rozwojowi pędów towarzyszyło różnicowanie się elementów drewna i łyka. Liście roślin hodowanych na pożywkach zawierających cytokininę BA zbudowane były z większej liczby warstw komórek miękiszu asymilacyjnego. Rozmiary aparatów szparkowych były u roślin hodowanych na pożywkach z dodatkiem cytokininy mniejsze, czemu towarzyszyły analogiczne zmiany w wielkości komórek właściwych skórki. Na krzacasty pokrój roślin spowodowany obecnością cytokininy w pożywce złożyło się zwiększenie grubości i szerokości liści oraz rozrost rdzenia łodygi przy jednoczesnym zahamowaniu wzrostu elongacyjnego komórek. Auksyna IBA nie sprzyjała rozwojowi istniejących pędów bocznych. Stymulowała wzrost elongacyjny rdzenia łodygi. Aparaty szparkowe roślin obu gatunków hodowanych na pożywkach z dodatkiem auksyny były większe. Stwierdzono zwiększenie częstotliwości występowania dwujądrowych komórek pod wpływem auksyny IBA oraz cytokininy BA u obu badanych gatunków w komórkach miękiszowych łodygi i liścia.

SŁOWA KLUCZOWE: regulatory wzrostu, IBA, BA, anatomia, *Bromeliaceae*, in vitro, pożywka RM.