ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XL — nr 2
1971

Development of new plants from leaves and roots of *Atropa belladonna* L. in the *in vitro* culture

M. A. ZENKTELER

Department of General Botany, Adam Mickiewicz Univeristy, Al. Stalingradzka 14, Poznań, Poland

(Received: December 19, 1970.)

Abstract:

Isolated leaves, leaf fragments and fragmented roots of *Atropa balladonna* were grown *in vitro* in order to study their morphogenetic potentials. In the presence of kinetin and IAA, a great number of shoot buds arose from the leaves and roots. In those cases when cut leaves were implanted a callus arose from the leaves which subsequently gave rise to many buds. In the absence of kinetin and IAA buds were not formed. In the presence of IAA only numerous roots arose from the cut ends of the leafe stalk and from the fragmented root.

INTRODUCTION

Reports on regeneration of whole plants from tissues and organs cultured in vitro were numerous during the past few years. Cells of various tissues of the mature plant can give rise to embryos or shoot buds e.g. the phloem cells of carrot (Steward et all. 1958), pith of tabacco stem (Skoog and Miller, 1957), young floral primordia of *Ranunculus* (Konar and Nataraja, 1964), anthers of *Datura* (Guha and Maheshwari, 1964) and *Nicotiana* (Kazuo Nakata and Masao Tanaka, 1968), nucellar tissue of *Citrus* (Rangaswamy, 1961), immature embryos of *Fagopyrum* (Zenkteler, 1966), embryos of *Solanum* (Takuzo Yamada et all. 1967), hypocotyl of *Asparagus* (Wilmar and Hellendoorn, 1968), leaves of *Nicotiana* (Gupta et all. 1966). This is not a complete list, but it suffice to indicate the range of organs and tissues which in artificial conditions are capable to produce shoot buds or embryos.

Bud initiation in callus tissue, particularly in response to various nutrient media, has also been studied by several authors (Cutter, 1965; Torrey, 1966). However, there are only few examples of in vitro for-

mation of embryos and buds in leaves of flower plants. Among these are: the palisade cells of *Macleya cordata* (K o h l e n b a c h, 1965), *Nicotiana tabacum* (G u p t a, 1966 and the epidermal cells of *Dendrophtoe falcata* (N a g and J o h r i, 1970).

The formation of buds directly from the roots of *Convolvulus arvensis* cultured *in vitro* is discussed extensively by B o n n e t and T o r r e y (1966). B u t c h e r and S t r e e t (1964) give a list of 72 species, the excised roots of which were cultured *in vitro*. The roots of *Nicotiana glauca* were the only ones which after more than one year of growth showed a tendency to form shoots.

Isolated roots, root callus, stem and leaf callus of *Atropa belladonna* L. were investigated in *in vitro* culture by W e s t and M i k a. (1967). B h a n d a r y et al. (1969) established from seedlings of *Atropa belladonna* root, callus and cell suspension cultures. Recently T h o m a s and S t r e e t (1970) studied organogenesis in cell suspension cultures of this species.

In the present paper the direct differentiation of shoot bouds from *in vitro* formed leaves and root segments is reported.

MATERIAL AND METHODS

Dicotyledonous embryos from the ovules of *Atropa belladonna* were inoculated under aseptic conditions on L i n s m a i e r and S k o o g medium (1965) with kinetin — 4 mg/l and IAA — 2 mg/l. The cultures were kept under diffuse light at 24 ± 2°C. The embryos produced callus which differentiated and formed many shoot buds. Single shoots were isolated and transferred on the same medium only with kinetin omitted (preliminary experiments howed that kinetin inhibited the production of roots) in order to obtain normally developed seedlings. Leaves (whole or cut into two transverse parts) about 3 cm in length and about 0,8 cm fragments of roots obtained from seedlings grown *in vitro* were used as the experimental material. This material was inoculated on Linsmaier and Skoog medium in 3 combinations: A. without kinetin and IAA; B. with IAA — 2 mg/l; C. with kinetin — 4 mg/l and IAA — 2 mg/l. Explants on the day of inoculation were fixed in FAA. Fragments of explanted leaves and roots were fixed after 2, 4, 6 and 10 weeks of culture. The standard paraffin method was used for investigating the major steps in the anatomy of bud formation.

OBSERVATIONS

The dicotyledonous embryo grew directly into a young plant on „A" and „B" medium. On „C" medium 10—12 days after inoculation a callus was formed from the embryo (Fig. 1). The callus consisted of cells of

various size. After the next 10 days several centres of smaller isodiametric cells were observed in the callus. These isodiametric cells showed a high mitotic activity, and consequently became organized into meristematic zones resembling bud primordia. During the next week numerous shoots arose from the callus (Fig. 2). The morpohogenic potentials of the callus were very high. The size and number of the growing buds and shoots differed in relation to the period of culture. It was found that 6 weeks after the embryos were inoculated, about 50—80 shoot buds of various size were present (Figs. 3, 4). When leafy shoots developed from buds were transferred on a fresh "C" medium they multiplied and produced many new shoots (Fig. 5). However, when they were transferred on "A" or "B" medium they produced normally developed seedlings (Fig. 6). Leaves and segments of roots obtained from seedlings grown on "B" medium were used as experimental material for further investigations.

I. Differentiation of buds from leaves

Excised leaves (Fig. 7) about 3 cm in length or cut into two transverse parts were implanted in such a way that the whole lamina was in contact with the nutrient medium. When whole leaves were transferred on "A" medium, no shoot buds were formed even after 10 weeks of culture. After 4—6 weeks of culture, the leaves became larger but after the next 4 weeks they shrivelled, withered, and consequently the green colour vanished. As a rule, the fragmented leaves shrivelled and died much earlier than the whole ones.

On "B" medium, after 3 weeks of culture, roots appeared in close proximity to the cut ends of the leaf stalks (Fig. 8). Similarly as on "A" medium, no bud formation was observed even after a period of 7 weeks of culture.

On "C" medium the leaves behaved completely differently. After 2—3 weeks the palisade layer proliferated and formed several small outgrowths composed of isodiametric cells (Fig. 9). This indicated that the palisade cells underwent repeated transverse divisions. In these outgrowths several meristematic zones resembling shoot apices were organized (Fig. 10). About 5 weeks after inoculation numerous shoots developed from various points on the leaf surface (Fig. 11). The cut portions of the leaves produced poorly growing hard callus from which subsequently many shoots arose (Fig. 12). When parts of the callus were subcultured on a fresh medium they also produced new shoots.

The majority of shoot buds were formed on the surface of the leaf which was not in direct contact with the medium, however, in few instances they differentiated on both surfaces of the leaf. The number of shoot buds formed per cultured leaf ranged from 8 to 18.

Very young (less than 1 cm in length) and mature (more than 5 cm in

length) leaves behaved quite differently. The young ones never formed shoots even after 6 weeks of culture. The tendency to produce shoots in mature leaves was much higher than in the young ones, however, wounding or cutting of the leaf had a strong effect on the distribution and number of shoot buds formed. In most instances buds differentiated from callus which had developed in close vicinity to the wounded regions.

When shoots developed from leaves were cultured on "C" medium for more than 7 weeks, they started to form roots. In several cases shoots were transfered on "B" medium in order to enhance the production of roots and in those instances seedlings were even obtained after 5 weeks of culture. Chromosome counts showed that the shoot buds developed from leaves were diploid (2n = 72). The 8-week old seedlings, after a thorough washing in water, were transfered to soil in pots and raised in the culture room (Fig. 13).

II. Differentiation of buds from roots

Root segments (Fig. 14) of about 1,5 cm length obtained from seedlings grown on "B" medium were cultured on "A", "B", and "C" medium. As the transverse section revealed, the implanted roots possessed a diarchic structure. The single-layered endodermis was surrounded by a several layered cortical zone composed of parenchymatic cells (Fig. 15). The roots were smooth, white and slightly curved. The explanted root segments on "A" and "B" medium showed, mainly elongation growth, with only a little increase in diameter. On "B" medium, after 2 weeks of

---

Plate I

Fig. 1. Portion of an embryo (em) producing callus tissue after 12 days of culture on C medium. × 60.

Fig. 2. Shoot buds arising from callus tissue after 26 days of culture on C medium.

Figs. 3. 4. Development of shoots from callus tissue after 6 weeks of culture on C medium; on Fig. 3 a part of the buds was removed to show the callus tissue (arrow).

Fig. 5. Single shoot as shown on Fig. 4 (b) when transferred on C medium produced many new shoots after 4 weeks of culture. × 0,4.

Fig. 6. Normally developed seedling obtained from a single shoot (Fig. 4, b) which was transferred on B medium after 3 weeks of culture.

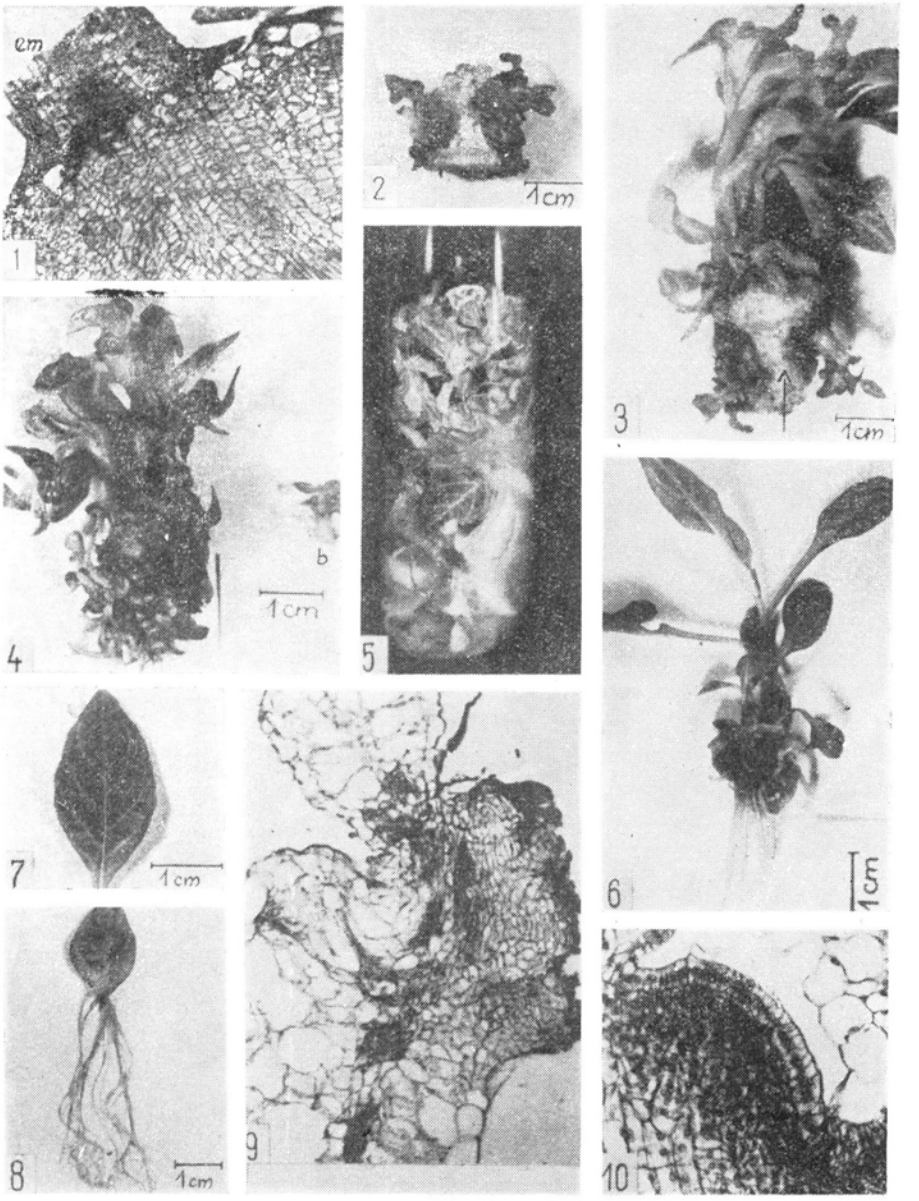
Fig. 7. Leaf at the time of inoculation.

Fig. 8. Leaf showing rooting on B medium.

Fig. 9. Transection of a leaf showing proliferation of pallisade cells after 2 weeks of culture on C medium. × 45.

Fig. 10. Differentiation of buds from the proliferated pallisade cells after 3 weeks of culture. × 90.

Plate I



culture lateral roots started to grow. On "A" medium lateral roots appeared only after more than 5 weeks of culture.

During the first week, the growth of excised roots on "C" medium was somewhat similar as on "A" and "B" medium. After the next 2 weeks, however, the roots increased in diameter and several days later started to proliferate producing tissue masses resembling nodules from which after the next 2 weeks shoot buds developed (Fig. 16). It was found in cross sectioned 3-week old roots that the parenchymatic cells of the cortical zone underwent radial enlargment and tangential division. The number of xylem cells increased enormously and the central part of the root was occupied by a group of lignified cells. In many areas of the peripher al layer of the cortical zone, nests of isodiametric cells were peripheral layer of the cortical zone, nests of isodiametric cells were the shoot apices differentiated (Fig. 18). Shoots which developed from explanted roots when transferred on "B" medium produced roots. Chromosome counts showed that buds obtained from fragmented roots were diploid.

DISCUSSION AND CONCLUSIONS

Bud initiation in callus tissue and in various organs cultured *in vitro* are controlled by chemical and physical factors of the environment. Most extensive studies on bud initiation in *Nicotiana tabacum* stem callus under the influence of an appriopriate auxin-kinetin balance were performed by Skoog and Miller (1957). Leaves of *Atropa belladonna* cultured on a medium without kinetin and IAA did not form buds. Similarly, on a medium containing only IAA buds were never formed. The ability of differentiation of buds appeared only when both kinetin and IAA were added to the medium. The present findings revealed that

---

Plate II

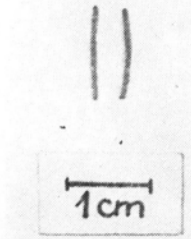
Fig. 11. Shoots arising from a leaf after 5 weeks of culture.
Fig. 12. Shoots arising from the callus tissue which was produced by the cut end of the leaf.
Fig. 13. Seedling obtained from the shoot produced in a leaf.
Fig. 14. Segments of roots at the time of inoculation.
Fig. 15. Transection of a root at the time of inoculation. × 100.
Fig. 16. Shoot buds arising from the root segment after 6 weeks of culture.
Fig. 17. Transection of a 3 week-old culture of the root segment; in the peripheral layer of the cortical zone nests of meristematic cells; the central part occupied by lignified cells. × 70.
Fig. 18. Shoot apices differentiated from the meristematic cells of the cortical layer after 5 weeks of culture. × 70.

Plate II

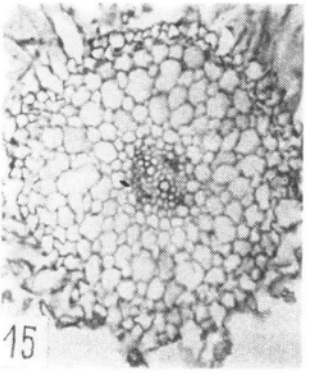
11

12

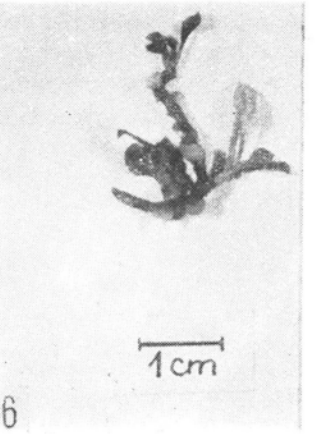
13



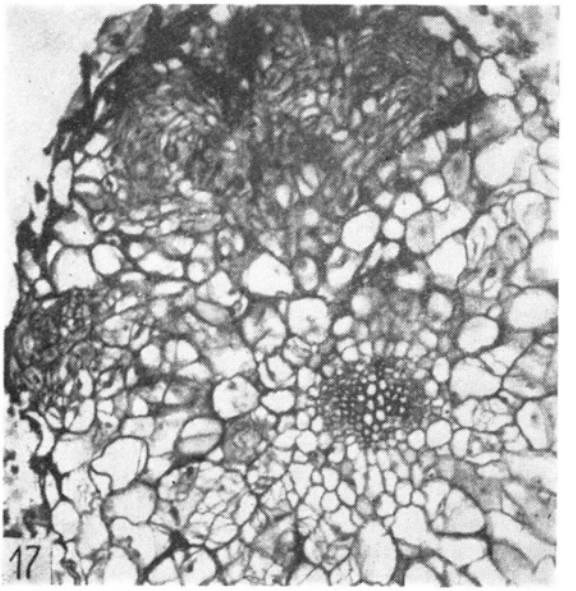
14



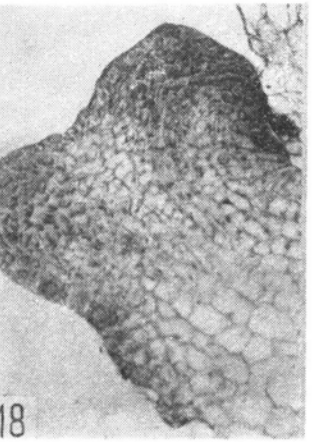
15



16



17



18

the *in vitro* formed leaves of *Atropa* are suitable material for investigating the role of cytokinins in shoot bud formation. Kinetin also stimulated bud formation in the leaves of *Nicotiana tabacum* (Gupta et al. 1966) and *Dendrophtoe falcata* (Nag and Johri, 1970), however, the leaves of *Nicotiana* formed buds only when coconut milk was added to the medium.

The meristematic activity of root segments was associated with the formation of lateral roots and buds. Buds were formed only on a medium containing kinetin and they originated from many areas of the peripheral layer of the cortical zone. In bud formation in *Convolvulus* (Torrey, 1966), only pericycle cells around protoxylem participated in the primordial initiation.

The studies of West and Mika (1957), Bhandary et al. (1969), Thomas and Street (1970) have shown that callus obtained from roots, stems and leaves of *Atropa belladonna* reveals high morphogenetic potentials. The callus cells regenerate and produce normally developed seedlings. The cell suspension cultures are also able to form aggregates of cells which give rise under appriopriate conditions of culture to shoots and embryo-like structures.

I wish to thank the Head of the Department of General Botany Prof. Dr A. Szweykowska for the facilities and for critical reading of this paper.

REFERENCES

Bhandary S. B. R., Collin H. A., Thomas E., and Street H. E., 1969, Root, callus and cell suspension cultures, from *Atropa belladonna* L. and *Atropa belladonna*, cultivar *lutea* Döll., Ann. Bot. 33 (132): 647—656.

Bonnet H. T., Torrey J. G., 1966, Comparative anatomy of endogenous bud and lateral root formation in Convolvulus Arvensis roots cultured *in vitro*, Am. J. Bot. 53 (5): 496—507.

Butcher D. N., Street H. E., 1964, Excised root culture, Bot. Rev. 30 (4): 513—536.

Cutter E. G., 1965, Recent experimental studies of the shoot apex and shoot morphogenesis, Bot. Rev. 31 (1): 7—113.

Guha S., Maheshwari S. C., 1964, In vitro production of embryos from anthers of Datura, Nature 204 (4957): 497.

Gupta G. R. P., Guha S., Maheshwari S. C., 1966, Differentiation of buds from leaves of *Nicotiana tabacum Linn.* in sterile culture, Phytomorphology 16 (2): 175—182.

Kazuo Nakata., Masao Tanaka, 1968, Differentiation of embryoids from developing germ cells in anther culture of tobacco, Jap. J. Genet. 43 (1): 65—71.

Kohlenbach, H. W., 1965 Über organisierte Bildungen aus Macleaya cordata kallus, Planta 64: 37—40.

Konar R. N., Nataraja K., 1964, In vitro control of floral morphogenesis in *Ranunculus sceleratus*, Phytomorphology 14: 558—563.

Linsmaier, E. M., Skoog F., 1965, Organic growth factor requirements of tobacco tissue culture, Physiol. Plantarum 18: 100—127.

Nag K. K., Johri B. M., 1970, Effects of cytokinins and injury on the formation of shoot buds by leaves of Dendrophthoe falcata, Planta 90: 360—364.

Rangaswamy N. S., 1961, Experimental studies on female reproductive structures of *Citrus microcarpa* Bunge, Phytomorphology 11: 109—127.

Skoog F., Miller C. O., 1957, Chemical regulation of growth and organ formation in plant tissues cultured in vitro, Symp. Soc. Exptl. Biol. 11: 118—131.

Steward F. C., Mapes M. O., Smith J., 1958, Growth and organized development of cultured cells. 1. Growth and division of freely suspended cells, Am. J. Bot. 45: 633—703.

Takuzo Yamada., Hiroko Nakagawa., 1967, Studies on the differentiation in cultured cells. I. Embryogenesis in three strains of *Solanum* callus, Bot. Mag. Tokyo 80: 68—74.

Thomas E., Street H. E., 1970, Organogenesis in cell suspension cultures of *Atropa belladonna* L. and *A. belladonna* cultivar *lutea* Döll., Ann. Bot. 34 (136): 657—669.

Torrey J. G., 1966, The initiation of organized development in plants, Adv. in Morphogenesis 5: 39—91.

West F. R., Mika E. S., 1957, Synthesis of atropine by isolated root and root — callus cultures of Belladonna, Bot. Gaz. 119 (1): 50—54.

Wilmar C., Hellendoorn M., 1968, Growth and morphogenesis of *Asparagus* cells cultured in vitro, Nature 217: 5126.

Zenkteler M., 1966, Multiple shoot formation from the embryonal mass of *Fagopyrum sagittatum* Gilib., Cur. Sci. 35: 18—19.

Powstawanie roślin z liści i korzeni Atropa belladonna w hodowli in vitro

Streszczenie

Zarodki *Atropa belladonna* hodowano w warunkach sterylnych na pożywce Linsmaiera i Skooga z dodatkiem kinetyny 4 mg/l i IAA 2 mg/l. Zarodki wytwarzały tkankę kalusową z której następnie powstawały liczne pąki. Pąki te odcinano i przenoszono na pożywkę bez dodatku kinetyny, na której wykształcały się w pełni rozwinięte siewki. Z siewek tych odcinano: liście w całości (około 3 cm długości), tej samej wielkości liście przecięte w poprzek na dwa fragmenty oraz fragmenty korzeni około 0,8 cm długości i przenoszono do pożywki Linsmaiera i Skooga w trzech kombinacjach: A) bez kinetyny i IAA, B) z dodatkiem IAA — 2 mg/l, C) z dodatkiem kinetyny — 4 mg/l i IAA — 2 mg/l.

Na pożywce „C" z całych liści oraz z fragmentów korzeni wyrastały liczne pąki. W przypadku fragmentowanych liści w miejscu cięcia najpierw powstawała tkanka kalusowa, w której następnie formowały się pąki. Na pożywce „B" liście wyszczepiane w całości, fragmenty liści oraz fragmenty korzeni wykazywały tylko rizogenezę. Na pożywce „A" nie dochodziło do powstawania pąków i korzeni. Z pąków liściowych i korzeniowych uzyskano na pożywkach „A" i „B" prawidłowo rozwinięte siewki.

Liczba chromosomów w siewkach wynosiła 72 (2n).