ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLVIII, nr 1: 47—53
1979

Comparison of auxin activity in tumourous and normal callus cultures from sunflower and tobacco plants

Z. CHIREK

Department of Plant Physiology, University of Łódź

(Received: August 2, 1978)

Abstract

In normal and tumourous calluses of sunflower and tobacco the level of extractable auxins was determined by *Avena coleoptile* straight growth test.

Auxin activity was detected practically in two zones: I — at position with R_f 0.2-0.4 and II — at position with R_f 0.6-0.9. The tumour tissues of sunflower and tobacco plants, representing different types of neoplastic growth exhibit a 3 times higher auxin activity as compared with that of the corresponding normal tissues.

Tobacco tissues, on the other hand, had a higher auxin level than the corresponding sunflower tissues and they exhibited different proportions in the activity of zones I and II, which points to a dominance of genetic regulation of hormone metabolism in these plants.

INTRODUCTION

Tobacco and sunflower plant tumours represent two different types of neoplastic growth — known as teratoma and inorganised growth, characteristic for polysomatic and nonpolysomatic plants, respectively. Characteristic of teratoma is growth in numerous centres, the presence of giant cells, differences in the sizes of nuclei, and an increased DNA content. The inorganised type of tumour occurring in sunflower plants contains minute even-sized cells with diploid nuclei similarly as does the mother plant (K u p i l a, 1958).

Phytohormones, and among them auxins, play an important role in tumour growth. The formation of giant cells with flake-like nuclei in the tumourous tissues was attributed to the locally changing auxin concentrations within these tissues (R a s h, S w i f t, K l e i n, 1959; K u p i l a - A h v e n n i e m i, 1968).

The results of Butcher et al. (1975) studies and the data of other authors indicate that sunflower tissues are more stable when cultured in vitro as compared with those of polysomatic plants which exhibit various degrees of polyploidy, depending on the kind and amount of growth regulators applied, mainly those of the auxin group (Dons et al., 1974).

No data were found in available literature concerning comparison of endogenous growth regulators in inorganised and teratoma type tumours, whereas their anatomical-cytological aspect has been described in detail.

It seemed, therefore, useful to compare the auxin level in normal and tumorous tissues of sunflower and tobacco, that is of plants representing different types as regards to the occurrence of somatic polyploidy and the types of tumours.

MATERIAL AND METHODS

The material consisted of tissue colonies of normal and tomourous (crown-gall type) calluses of *Nicotiana tabacum* L. c. 'White Burley' and *Helianthus annuus* L. cv. 'Borowski prążkowany'. Solid Murashige and Skoog medium (1962) modified by Linsmaier and Skoog (1965) supplemented with 50 mg/dm^3 of casein hydrolysate for both sunflower tissues and with growth regulators (IAA — 2 mg/dm^3, kinetin — 0.25 mg/dm^3) in the case of normal calluses was used. pH of the medium was adjusted to 5.6 for tobacco tissues and 5.9 for those of sunflower. About 250-mg fragments of tissues were inoculated into the sterile medium in test tubes and cultured in an incubator at 25±1°C under continuous fluorescent illumination of daylight type for 33-35 days.

Auxin was extracted from 10-g or/and 5-g tissue portions after Bayer (1967, 1968), the extraction time was, however, reduced from 20 to 3-4 h. According to Hemberg (1972) 2-3 h of extraction are sufficient for obtaining free auxins.

The acidic ether extracts were separated on plates coated with silica gel (Kodak) in the solvent system: butanol-water-ammonia (10:10:1 v/v, upper phase) and tested for the auxin activity.

The biotest and calculation of results. The *Avena coleoptile* straight growth test was applied. It was run on Petri dishes with 3 ml of the test solution after Nitsch (1956) with the use of 8-10 (10 mm) oat coleoptile segments after Bentley (1962). The results as per cent of increment of the control (on the blank segment of the chromatogram) are shown in the form of separate histogram for each determination. Standard IAA in 0.01-1 μg/cm^3 concentrations was analogously tested after development and elution of the chromatogram. On the basis of

Table 1

Auxin activity in the sunflower and tobacco tissue cultures

Plant	Sunflower (*Helianthus annuus*)						Tobacco (*Nicotiana tabacum*)						
Tissue	Callus			Tumour			Callus			Tumour			
Zone of activity (R_f)	I 0.1—0.4	II 0.5. 0.6 —0.8 .0.9	total	I 0.1—0.4	II 0.4, 0.5— 0.9, 1.0	total	I 0.2—0.4	II 0.5, 0.6— —1.0	total	Ia 0— —0.2	Ib 0.2, 0.3—0.4	II 0.5, 0.6—0.8, 1.0	total
µg-equ. IAA/kg fresh weight (±s.e.)	17 ±5.7	14 ±2	31 ±7.5	13 ±2	33 ±3	46.5 ±3.5	12 ±3	23 ±5	35 ±6.3	12 ±1	29 ±7.5	60 ±18.8	101 ±19
µg-equ. IAA/g dry matter			0.4			1.2			0.6				1.5

several series of results a standard curve was plotted from which the auxin activity of the particular chromatogram segments was expressed as μg-equivalents of IAA/cm³ of the test solution. A similar method of the results reading was applied by Eliasson for ABA (1975).

The confidence interval was calculated after Student for the control sample. It amounted to ±17 per cent of the control increment value. Readings of the auxin activity were taken only for those chromatogram segments for which the increment in the test exceeded the latter value.

For each kind of tissue 5-6 series of determinations were performed. The mean results and standard error related to 1 kg of fresh tissue are listed in the table.

RESULTS AND DISCUSSION

The sunflower tumour tissue exhibited a loose granular structure, a high weight increment and a high degree of hydratation. After 5 weeks of culture one colony weighed on the average 2750 mg and contained about 3.8 per cent of dry matter.

Normal callus tissue was compact and hard and its rate of growth was slower (mean weight of 1 colony 1410 mg, dry matter content 7.8%). Both sunflower tissues were greenish in colour.

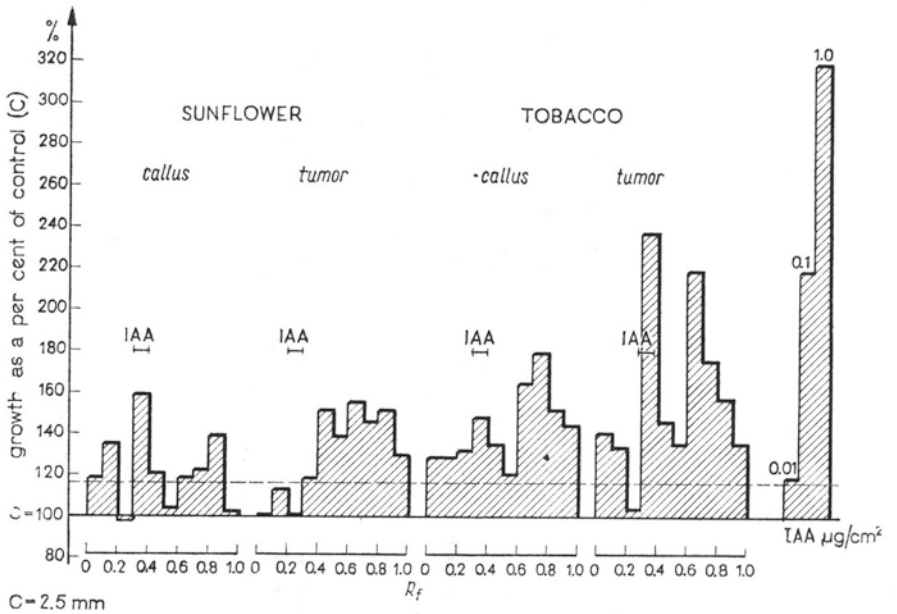

Fig. 1. Histogram of auxin activity of ether extracts of sunflower and tobacco calluses and tumours in bioassay of *Avena coleoptile* straight growth. Dotted line indicates confidence limit (95 %) for control.

The tobacco tissues were whitish-cream coloured, convex, with a fluffy surface. The tumour tissue grew somewhat slower than the normal one, but had a higher dry weight. Five-week tumour colonies reached a weight of 1530 mg (mean) with a dry matter of 6.6 per cent, while normal callus tissues weighed 1880 mg and their dry matter was 5.8 per cent.

Auxin activity in acidic ether extracts from the particular tissues is presented as a per cent increment of the control on the histogram (figure) which gives the results of a single experiment and in the table as μg-equivalents of IAA (mean of 5 analyses). The table gives also the range of R_f values for particular active zones of the chromatograms.

In normal sunflower callus the contribution of auxin activity in zones I and II is 55 and 45 per cent, respectively. In sunflower tumour the activity of zone I reaches maximum at R_f 0.3-0.4, 0.5. Contrary to normal callus, zone II exhibits a higher activity (72% of total). In reference to fresh weight total activity is higher by 50 per cent in sunflower tumour tissue as compared to that in normal callus, and if the different degree of hydratation is taken into account it is 3 times higher in the tumour.

In normal callus of tobacco zone I of activity shows a maximum at R_f 0.2-0.4, and zone II at positions 0.5, 0.6-1.0 R_f exhibits twice higher activity than that of zone I. In tobacco tumour tissue 3 peaks of activity occur, the activity at 0.2, 0.3-0.4 R_f (Ib) constituting 30 per cent of total, and at 0.5, 0.610.8, 1.0 R_f (II) 60 per cent of total. Total auxin activity in the extract from tobacco tumour tissue is about 3 times of that in normal callus, both in relation to fresh and to dry weight.

Auxin activity of zone I in the range 0.2, 0.3-0.4 R_f corresponds to the position of standard IAA. Bayer (1969) identified under similar separation conditions the presence of IAA in this zone in the shoots of several tobacco species, and Syono and Furuya (1972) in the callus tissue of tobacco. It would seem that in the tissues examined in the present study this auxin also occurs. In the case of callus tissues it is probably uptaken from the medium.

In tumourous tissues a higher activity is noted in zone II. Bayer (1969) demonstrated by gas chromatography the occurrence of large quantities of indole-3-butyric acid (IBA) at the position with 0.45-0.65 R_f in tobacco tumour forming hybrids.

As regards to the kinds of auxin in tumourous tissues, data are available in literature only for the 1950s and 1960s, suggesting the occurrence of IAA, indole-3-acetonitrile (IAN), ethyl IAA ester (IAE) and indole-3-carboxylic acid (ICA). The results of quantitative determinations point in general to a higher (up to 10 times) auxin content in tumour tissues as compared with that in normal ones, however, the data

of various authors are discrepant (Sequeira, 1973; Lippincott, 1975).

The present results made possible the determination of the level of compounds with auxin activity in normal and tumourous tissues of sunflower as 31 and 45 μg-equ. of IAA/kg of fresh weight, respectively, the corresponding values for tobacco being 35 and 100 μg-equ. of IAA/kg of fresh weight. If the different degree of hydratation is taken into account, both tumour tissues contained 2.5-3 times more auxin-like compounds as compared with the homologous callus tissues. Thus, independently of the type of the tumour, a high activity of biosynthetic auxin systems seems to characterise tumours in general.

Comparison of tobacco and sunflower tissues, that is those of plants representing polysomatic and nonpolysomatic genomes, indicates a higher auxin level in both tobacco tissues. It seems that the genetic regulation of hormonal metabolism characteristic for these two plant species subsists independently of the tissue type. This is also indicated by the different proportion of auxin activity in zones I and II, which suggests certain qualitative differences.

The foregoing suggestion may serve as outset point for further investigations aiming at identification of the particular compounds by appropriate methods.

REFERENCES

Bayer M. H., 1967. Thin-layer chromatography of auxin and inhibitors in *Nicotiana glauca*, N. langsdorffii and three of their tumor-forming hybrids. Planta (Berl.) 73: 329-337.

Bayer M. H. and M. R. Ahuja, 1968. Tumor formation in *Nicotiana:* Auxin levels and auxin inhibitors in normal and tumor-prone genotypes. Planta (Berl.) 79: 292-298.

Bayer M. H., 1969. Gas chromatographic analyse of acidic indole auxins in *Nicotiana*. Plant Physiol. 44: 267-271.

Bentley J. A., 1962. Analysis of plant hormones. In: Methods of Biochemical Analysis, vol. IX: 75-121. Ed. by David Glick. Intersciences Publishers, John Wiley Sons, New York, London.

Butcher D. N., A. K. Sokege, I. C. Tommerup, 1975. Factor influencing changes in ploidy and nuclear DNA levels in cells from normal, crown-gall and habituated cultures. Protoplasma 86: 295-308.

Dons J. J. M., M. Valentijn, R. A. Schilperoort and P. van Duijn, 1974. Nuclear DNA content and phytohormone requirements of normal, crown-gall and habituated tissues of *Nicotiana tabacum* var. White Burley. Exp. Cell Res. 89: 283-294.

Eliasson L., 1975. Effect of indoleacetic acid on the abscisic acid level in stem tissue. Physiol. Plant. 34: 117-120.

Hemberg T., 1972. The effect of kinetin on the occurrence of acid auxin in *Coleus blumei*. Physiol. Plant. 26: 98-103.

Kupila S., 1958. Anatomical and cytological comparison on the development of crown-gall in three host species. Ann. Bot. Soc. "Vanamo" 3 (1): 1-89.
Kupila-Ahvenniemi S., E. Therman, 1968. Morphogenesis of crown-gall. Adv. Morphogen. 7.
Linsmaier E. M., F. Skoog, 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18: 110-127.
Lippincott J. A., B. B. Lippincott, 1975. The genus Agrobacterium and plant tumorigenesis. Ann. Rev. Microbiol. 29: 377-405.
Murashige T., F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
Nitsch J. P., 1956. Methods for the investigation of natural auxins and growth inhibitors. In: The chemistry and Mode of Action of Plant Growth Substances: 3-31. Ed. by R. L. Wain and F. Wightman, London.
Rash E., Swift H., R. M. Klein, 1959. Nucleoprotein changes in plant tumor growth. J. Biophysic. Biochem. Cytol. 6 (1): 11-34.
Sequeira L., 1973. Hormone metabolism in diseased plants. Ann. Rev. Plant. Physiol. 24: 353-380.
Syono K. and T. Furuya, 1972. Effects of cytokinins on the auxin requirement and auxin content of tobacco calluses. Plant and Cell Physiol. 13: 843-856.

Author's address:
Dr Zofia Chirek
Department of Plant Physiology
Institute of Physiology and
Cytology, University of Łódź
Banacha Str. 12/16, 90-237 Łódź; Poland

Poziom auksyn w kulturach kalusów tumorowych i prawidłowych słonecznika i tytoniu

Streszczenie

W kalusach normalnych i tumorowych słonecznika i tytoniu określano poziom ekstrahujących się auksyn metodą testu prostego koleoptyli *Avena*.

Aktywność auksynową stwierdzono w dwu zasadniczych strefach: I — w pozycji 0.2,0.3-0.4 R_f, odpowiadającą prawdopodobnie IAA, II — w pozycji 0.5, 0.6-0.9,1.0 R_f, zawdzięczaną prawdopodobnie innym kwasom indolowym oraz obojętnym pochodnym IAA.

Kalus prawidłowy słonecznika dysponował większą aktywnością w I strefie; w tumorze dominowała II strefa, a całkowita aktywność była wyższa w porównaniu do tkanki prawidłowej.

W obu tkankach tytoniu — przeważała aktywność auksynowa II strefy (60% całości), a tumor zawierał 3-krotnie więcej auksyn niż kalus normalny.

Wyższy poziom auksyn w tkankach tumorowych słonecznika i tytoniu — reprezentujących odmienne typy wzrostu neoplastycznego — potwierdza pogląd, że aktywacja biosyntezy auksyn jest cechą wzrostu tumorowego w ogóle.

Natomiast wyższy poziom auksyn w tkankach tytoniu w porównaniu do odpowiednich tkanek słonecznika wskazuje na dominację genetycznej regulacji gospodarki hormonalnej w tych roślinach.