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Indole Auxins in Spinach Plants Grown in Long and Short Days*

LJUBINKA ĆULAFIĆ and MIRJANA NEŠKOVIĆ

Institute of Botany, Faculty of Science, and Institute for Biological Research, Beograd**

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Abstract. The endogenous auxin-like substances were analyzed in the shoot extracts of young spinach seedlings, exposed to photoperiodic induction. At least eight indole auxins were found. One of them was identified as tryptophan, the other one is most probably IAA. The plants grown in long days had a higher level of ether soluble auxins than the controls in short days. Separate extractions of plants after each of the eight inductive days showed that the auxin content was not constant, but subjected to irregular oscillations. However, parallel oscillations were also found in control plants grown in short days. Staminate plants were found to contain more endogenous auxins than the pistillate ones. It is concluded that the quantitative changes in auxins during the photoperiodic induction are probably not related to flowering, but to some other growth process, common to all plants in that phase of growth. The higher level of auxins in staminate plants may be the cause of their faster elongation before the onset of flowering.

Spinach is a genotypically determined dioecious plant, with an obligate long-day requirement for flowering. The qualitative response to the adequate photoperiods and a relatively long induction period make this plant a good object for studying the endogenous hormonal factors involved in flowering and sex determination. It is well-known that auxins have a major role in the growth of vegetative plant organs, in the correlative phenomena and differentiation. Since the photoperiodic induction brings about the changes in the growth rate and differentiation pattern of the shoot apex, one could expect to find also some changes in the levels of auxins during the same period. The present paper describes the results of an investigation of endogenous auxin-like substances, obtained by extraction after each of the inductive long days.

Endogenous auxins of spinach have not been studied extensively so far. WILDMAN and BONNER (1947) found no free auxins in the stem extracts and studied only the so-called "bound" auxins. The conversion of tryptophan

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** *Address:* Takovska 43, 110 00 Beograd, Yugoslavia.

to auxin was studied in spinach by WILDMAN *et al.* (1947). They found that oxygen was required for the mechanism of tryptophan conversion to auxin, and that an intermediate compound containing a carbonyl group was formed during the conversion. The experiment suggested that indole-3-pyruvic acid may be the keto intermediate formed and that IAA might be then final product.

Material and Methods

Spinach (*Spinacia oleracea* L., cv. 'Matador') was sown in vermiculite and watered every 3rd day with Hoagland's nutrient solution. The plants were held in a phytotrone with the temperature regulated at 20 °C, the relative humidity at 75% and the illuminance at 12 000 lx. All the plants were maintained in short days of eight hours until the fourth leaf had developed. Then half of the plants were transferred to a similar phytotrone with continuous illumination for eight days.

Control plants, left in short days remained in the rosette stage, while the induced plants elongated and flowered within 14 days.

The plants were extracted during the induction period after each of the eight long day cycles. Twenty five plants were used for each extraction. An equal number of control plants grown in short days was extracted at the same time. Extractions were always done at nine in the morning. Whole plants, stems and leaves, were harvested, frozen with liquid nitrogen, crushed and extracted with cold methanol for 24 h at 3 °C. The methanol extract was shaken with light petroleum to remove the bulk of pigments, and evaporated to dryness. The residue was dissolved in 10 ml water, and extracted three times with ethyl ether at pH 3.0. Whole ether fraction was used for chromatography. The water fraction was neutralized and also used for chromatography after evaporation.

Preparative chromatography on Whatman 17 or 3 MM paper was used for further purification, with the following solvents: 1. n-propanol-water (3 : 1, v/v), 2. *i*-butanol-methanol-water (80 : : 5 : 15, v/v/v), 3. n-butanol-acetic acid-water (90 : 5 : 30, v/v/v), 4. n-butanol-ammonia (3 : 1, v/v). Active substances were located by oat first internode test (NITSCH and NITSCH 1956) and the indole compounds with reagents of Salkowsky, Ehrlich or Procházka. The corresponding zones were eluted, spotted on thin layers (0.25 mm) of silica gel H and developed in: 5. methyl acetate -*i*-propanol-ammonia (45 : 35 : 20, v/v/v), 6. ethyl acetate-*i*-propanol-water (65 : 24 : 11, v/v/v), 7. chloroform-methanol (93 : 7, v/v), 8. chloroform-ethanol (90 : 10, v/v), 9. chloroform-carbon tetrachloride-methanol (50 : 25 : 25, v/v/v), 10. *n*-propanol-5% ammonia (5 : 2, v/v), 11. *n*-butanol-acetic acid-water (2 : 1 : 1, v/v/v). Eluates from the silica gel were examined for fluorescence, using an Aminco-Bowman spectrophotofluorometer and then, after adding the buffered sucrose solution, tested with the oat first internode sections. Substances were also identified by measuring the pH-dependence of fluorescence intensity (BURNETT and AUDUS 1964). In some cases, the eluates were assayed with barley endosperm test to detect gibberellin-like substances (COOMBE *et al.*, 1967).

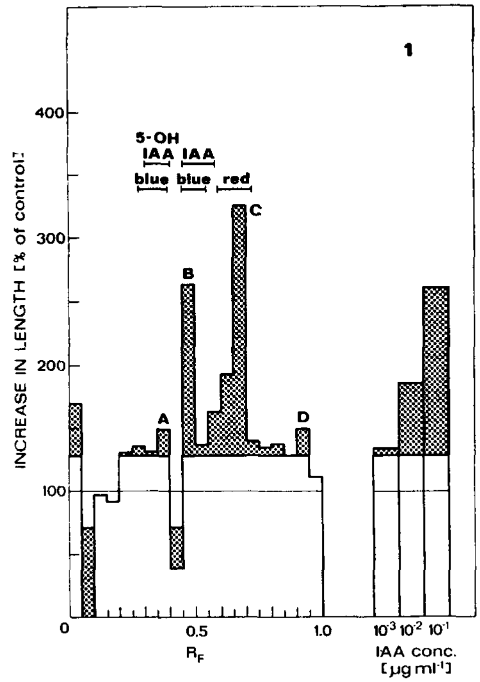
Results and Discussion

Before the extraction of growth substances it was necessary to establish precisely the photoperiodic behaviour of the plants used in the experiments. It was found that eight long days were necessary for the full flowering response at 20 °C. Five such days induce flowering in about 50% plants.

The bioassay of acid ether soluble fraction showed four zones of stimulation, giving also a positive reaction with the Ehrlich reagent. They were designated zones A, B, C and D respectively (Fig. 1).

Substance A, Rf 0.35—0.40, gives a blue-violet colour with Ehrlich reagent turning to blue a few hours later. The Rf of this substance corresponds in

Abbreviations used: IAA = indole-3-acetic acid, 5-OH IAA = 5-hydroxy-indole acetic acid, TLC = thin layer chromatography.



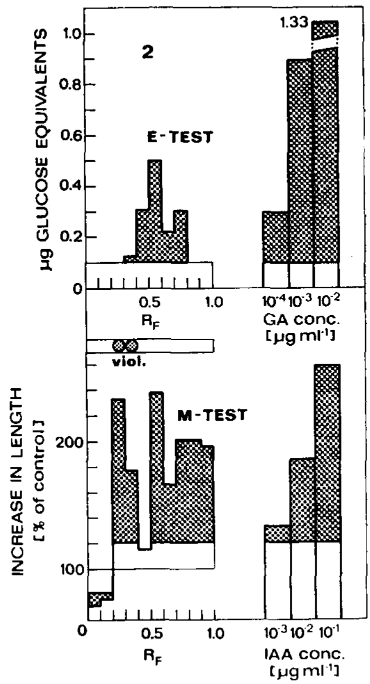


Fig. 1. Histogram of auxin-like activity in oat first internode test of ether soluble fraction, chromatographed on Whatman 3 MM paper. Solvent: n-propanol — water (3 : 1, v/v). Lines indicate the position of marker spots and colour reactions with Ehrlich reagent.

Fig. 2. Histograms showing the activity in barley endosperm test (E-test) and oat first internode test (M-test) of the substance C, eluted from the paper chromatogram (see Fig. 1). Chromatographed on a silica gel H layer, in ethyl acetate -i-propanol — water (65 : 24 : 11, v/v/v).

neutral solvent systems to the marker spot of 5-OH-IAA, but in acidic or alkaline solvents it is different and moves like a less acidic substance.

Substance B, Rf 0.45—0.55, gives a blue colour with Ehrlich. In four different solvent systems in paper chromatography it has a Rf value similar to IAA. The eluate of this zone, when applied to TLC, also shows an activity corresponding to IAA in five different solvent systems.

Substance C, Rf 0.60—0.80, gives a red-brown colour with Ehrlich and has a very high biological activity. Its Rf does not correspond to that of other indole compounds used as markers. Eluate of this zone applied to TLC, shows the presence of an active indole compound, but also a zone with gibberellin-like activity, which probably contributes to the high stimulation of paper chromatogram fractions (Fig. 2).

Substance D, Rf 0.90—1.0 has a low biological activity and gives no specific colour with Ehrlich reagent.

All the three substances, A, B and C, have the activation spectra at 290—295 nm, and the fluorescence spectra at 360—365 nm, which is characteristic of indole compounds.

The water soluble fraction also contains four Ehrlich positive substances designated E, F, G, H respectively (Fig. 3). One of them was identified as tryptophan, according to its chromatographic behaviour (solvents No. 5—9), activation-fluorescence spectra (290—360) and fluorescence intensity dependence on pH. Other substances from water fraction show different chromatographic properties from all the markers used and could not be identified by chromatographic methods.

The Change in the Level of Auxins during Photoperiodic Induction

The results of the quantitative determination of auxins were calculated by summing up the activities of substances A + B or A + B + C. In the latter case the activity of gibberellin-like component of zone C was also included. The total content of stimulating substances in 200 plants, expressed in μg equivalents of IAA was as follows: under long-day conditions substances A + B equaled to 0.18, A + B + C to 0.66 μg IAA. In short days the content of A + B was 0.09, that of A + B + C 0.22 μg equivalent IAA. It is clear that the total content of auxins is higher in long than in short days. In this respect our results are in agreement with the findings of other authors (CHAÏLAKHYAN and ZHDANOVA 1938).

The total content of auxins determined in each particular day of the inductive period was not constant, but showed rhythmic changes with irregular periods of 2—3 days (Fig. 4). Essentially the same oscillations were found in plants grown in long and short days. It seems unlikely, therefore, that these oscillations are inbolved in the induction of flowering.

For estimation of auxin-like substances in plants of different sex, plants were grown outdoors in normal summer days. While the stem apices were still vegetative, they were carefully cut off and frozen with liquid nitrogen. The plant stumps were labeled and left until the side branches developed, bearing visible flower primordia. The previously cut apices, belonging to

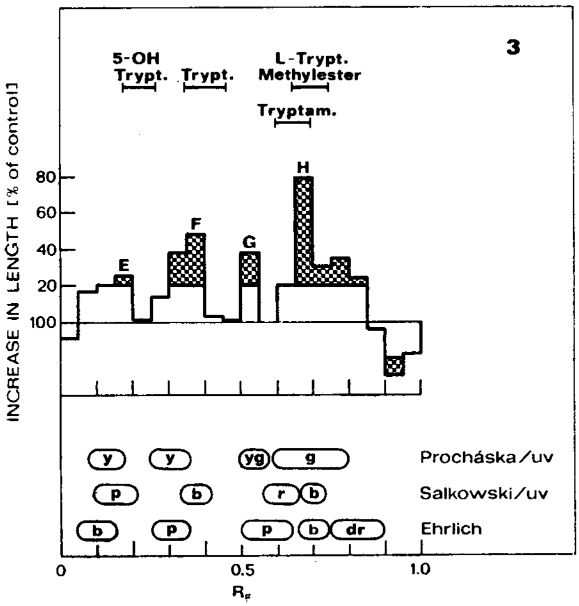
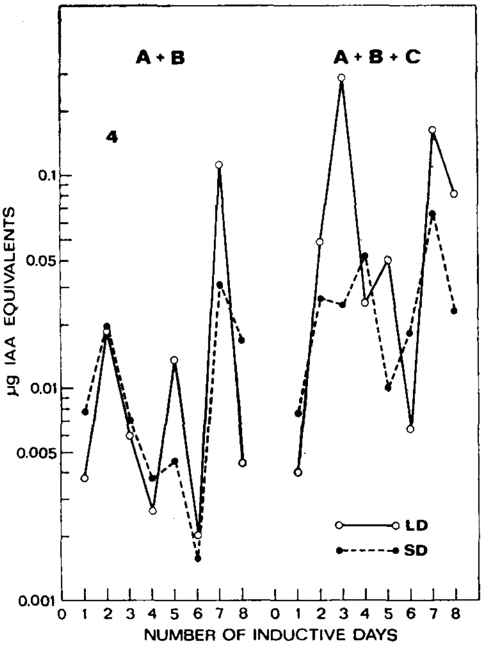


Fig. 3. Histogram of auxin-like substances in water soluble fraction, assayed by oat first internode test. Chromatographed on Whatman 3 MM paper. Solvent: n-propanol — water (3 : 1, v/v). Colours with reagents: *b* = blue, *p* = purple, *r* = red, *y* = = yellow, *yg* = yellow-green, *dr* = = dark red.



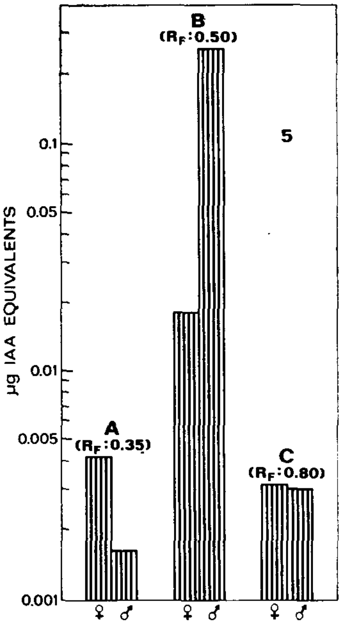


Fig. 4. Changes in the total content of auxin-like substances during the photoperiodic induction. Left: substances A + B, right: substances A + B + C. LD and SD: plants grown in long and short days respectively.

Fig. 5. The content of substances A, B and C in male and female plants, extracted 14 days after the photoperiodic induction.

male or female plants were then sorted out into two groups and extracted without thawing. In that way it was found that plants which were to become staminate (male) contained approximately two times more of auxin-like substance B, than the future pistillate (female) ones. Substance B is supposed to be IAA. A later extraction (14 days after the induction) of plants in which the sex of flowers way already visible, confirmed this result (Fig. 5).

From all these data it is possible to conclude the following:

Spinach stems and leaves contain at least eight indole compounds active in the biological test. One of them is tryptophan, the other one most probably IAA. The identity of other six compounds is unknown. Only the ether soluble substances showed significant changes during the induction.

The plants in long days contain more ether-extractable auxins than those under short day conditions. The total content of all ether soluble auxin-like substances is fluctuating during the induction, which is obviously related not to the flowering, but perhaps to some other process of growth or differentiation, such as development of leaves, for instance. In studying the changes in the level of auxin-like substances in long and short day plants during flower initiation, Nitsch and Nitsch (1965) also found that the content of growth substances was not constant and suggested that the changes could be the consequence of translocation from one to another stem part. Gilson (1957) did not find any difference in auxin level in *Hyoscyamus niger* under inductive or non-inductive conditions. Differences arise later on and are apparently correlated with the growth of flowers and fruits.

More auxin-like substances are found in staminate plants, both before and after the appearance of the visible signs of their sex. This seems to be at variance with some results obtained with other plants, which rather suggest that high auxin content is correlated with femaleness (HESLOP-HARRISON 1972). SLADKÝ (1972) also observed that the onset of pistillate flower differentiation was accompanied by a temporary rise of auxin level. However, a somewhat exceptional behaviour of spinach has already been noted. CHAÏLAKHYAN *et al.* (1969) found that exogenous gibberellic acid increases the number of female plants in long days, although in short days the same treatment induces flowering, but has no influence on the ratio of male to female plants. As pointed out by HESLOP-HARRISON (1972), it is still not clear whether the hormonal level found in a particular plant part and at a particular age is actually related to the development of flowers. The discrepancies in results and interpretations of different authors may also be due to different plant materials or methods used for investigation. As the male spinach plants elongate before flowering more than the female ones, it is also possible that the higher level of auxins is related to this process and not to the determination of sex.

Acknowledgement

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L. ĆULAFIĆ, M. NEŠKOVIĆ, Botanický ústav fakulty věd a Ústav pro biologický výzkum, Bělehrad, Jugoslávie: **Indolové auxiny v rostlinách špenátu pěstovaných při dlouhém a krátkém dni.** — Biol. Plant. **16** : 359—365, 1974.

V extraktech nadzemních částí klíčních rostlin špenátu vystavených fotoperiodické indukci byly stanoveny endogenní auxiny. Bylo zjištěno nejméně osm indolových auxinů. Jeden z nich byl identifikován jako tryptofan, další je s velkou pravděpodobností IOK. Rostliny rostoucí při dlouhém dni měly vyšší hladinu auxinů rozpustných v eteru než kontrolní rostliny rostoucí při krátkém dni. Jednotlivé extrakce rostlin vždy po osmi indukčních dnech ukázaly, že obsah auxinů není konstantní, ale že podléhá nepravidelným oscilacím. Paralelní oscilace však byly zjištěny i u kontrolních rostlin pěstovaných při krátkém dni. Samčí rostliny měly vyšší obsah endogenních auxinů než rostliny samičí. Ze zjištěných výsledků vyplývá, že kvantitativní změny v obsahu auxinů během fotoperiodické indukce pravděpodobně nejsou v žádném vztahu ke kvetení, ale k jiným růstovým procesům, společným všem rostlinám v této vývojové fázi. Vyšší hladina auxinů v samčích rostlinách pravděpodobně způsobuje jejich rychlejší prodlužování před nástupem kvetení.

BOOK REVIEWS

TROUGHTON, J. H., SAMPSON, F. B. **Plants. A Scanning Electron Microscope Survey.** — John Wiley and Sons Australasia Pty Ltd, Sydney, New York, London, Toronto 1973. 158 S. Geb. 5,60 £, brosch. 3,50 £.

Die durch das Rasterelektronenmikroskop aufgedeckten neuen Möglichkeiten zur Darstellung der Raumstruktur von Objekten wurden in vorliegendem Buche zur Bebilderung der Struktur verschiedener Pflanzenobjekte herangezogen (vergleiche ebenfalls TROUGHTON, J., DONALDSON, L. A.: Probing Plant Structure, Chapman and Hall, London, 1972; besprochen in Biol. Plant. **16** : 156, 1974). Auf 163 schwarzweissen unikaten Photographien sind die Raumstruktur von Pflanzenzellen, -geweben, die Innen- und Aussenstruktur von vegetativen und generativen Pflanzenorganen festgehalten. Die Objekte in diesem Bilderbuch der Pflanzenwelt sind taxonomisch angeordnet: das Buch umfasst Vertreter von Bakterien, Algen, Pilzen, Moosen, Farnen und Samenpflanzen, wobei die Objekte, die eine gute Bearbeitung durch das Rasterelektronenmikroskop ermöglichen wie z.B. Zellen von Diatomen, Samen, Pollenkörner, Gefässe, Blattoberflächen bevorzugt wurden. Jede Fotografie wird durch einen kurzen erklärenden Text begleitet, der ausser der Vergrösserung Angaben über die taxonomische Einreihung der Pflanze, deren Verbreitung, Besonderheiten ihrer Form, Struktur, bzw. physiologische Eigenschaften enthält, event. auf entsprechende Literatur hinweist. Das Buch enthält ein Pflanzenverzeichnis, ein Literaturverzeichnis und ein kurzes Sachregister. Es ist hauptsächlich für Hochschulen und Universitäten als Illustration botanischer und biologischer Texte bestimmt, es wird jedoch jeden Leser durch seine einzigartigen Fotografien packen und ihm neue Seiten der Pflanzenwelt, deren Raumstruktur enthüllen.

INGRID TICHÁ (*Praha*)

Sovremennye Problemy Fotosinteza [Gegenwärtige Probleme der Photosynthese]. — Izdatel'stvo Moskovskogo Universiteta, Moskva 1973. 213 S. Rbl. 1,73, russisch.

Dieses Buch ist dem 200. Jubiläum der Entdeckung der Photosynthese durch den englischen Gelehrten Joseph Priestley gewidmet und enthält elf Vorträge, die am 17. und 18. November 1971 in Moskau auf einer feierlichen Versammlung sowjetischer Wissenschaftler zu diesem Anlass vorgetragen wurden. Die Vorträge wurden als Übersichtsreferate zusammengestellt und behandeln historische sowie gegenwärtige Aspekte und Probleme der Photosynthese und Photosyntheseforschung (z.B. die Grundlagen der photosynthetischen Produktivität der Pflanzen, bioenergetischen Vorgänge der Photosynthese, photosynthetischen Pigmente, Photosynthesemechanismen und Primärvorgänge). Das Buch ist nur in russischer Sprache verfasst worden.

INGRID TICHÁ (*Praha*)