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Distribution of Tocopherols and Natural Growth Regulators in Etiolated Pea Seedlings

Rozmieszczenie tokoferoli i naturalnych regulatorów wzrostu
w etiolowanych siewkach grochu

Stowe and Obreiter (13) demonstrated a promoting effect of tocopherols on the growth of the stem of pea. They found that these compounds might be effective in cytochrome oxidation chain which supplies energy for the activity of auxin and gibberellin.

Baszyński and Maternowski (2) demonstrated an increased accumulation of α -tocopherol in the etiolated pea seedlings under the influence of IAA.

Recently, apart from papers on natural growth regulators in the development processes of plants, some reports have appeared on the effect of tocopherols in the control of flowering plants grown under non-inductive temperature and light conditions (3, 6, 7, 12).

The elucidation of the relationship between tocopherols and natural growth regulators depends upon the knowledge of their distribution in the etiolated seedlings. This is the aim of the present paper.

Studies on the distribution of natural growth regulators were carried out, but only a report by Gaunt and Stowe (10) has been published, so far, on the tocopherol distribution in pea seedlings.

MATERIAL AND METHODS

Pea seeds (*Pisum sativum* L. var. *Victoria*) were sterilized in 0.1% mercuric chloride solution for 30 min. and washed with distilled water several times. After the seeds had been soaked in water for 24 hrs., they were put into Petri dishes on filter paper. Germination took place in a thermostate at 23°C, in the dark. Samples were taken for analyses after 1, 3, 5, and 10 days of germination. One-day-old seedlings were examined totally and 3, 5, and 10-day-old seedlings were cut into sections, as shown in Fig. 1.

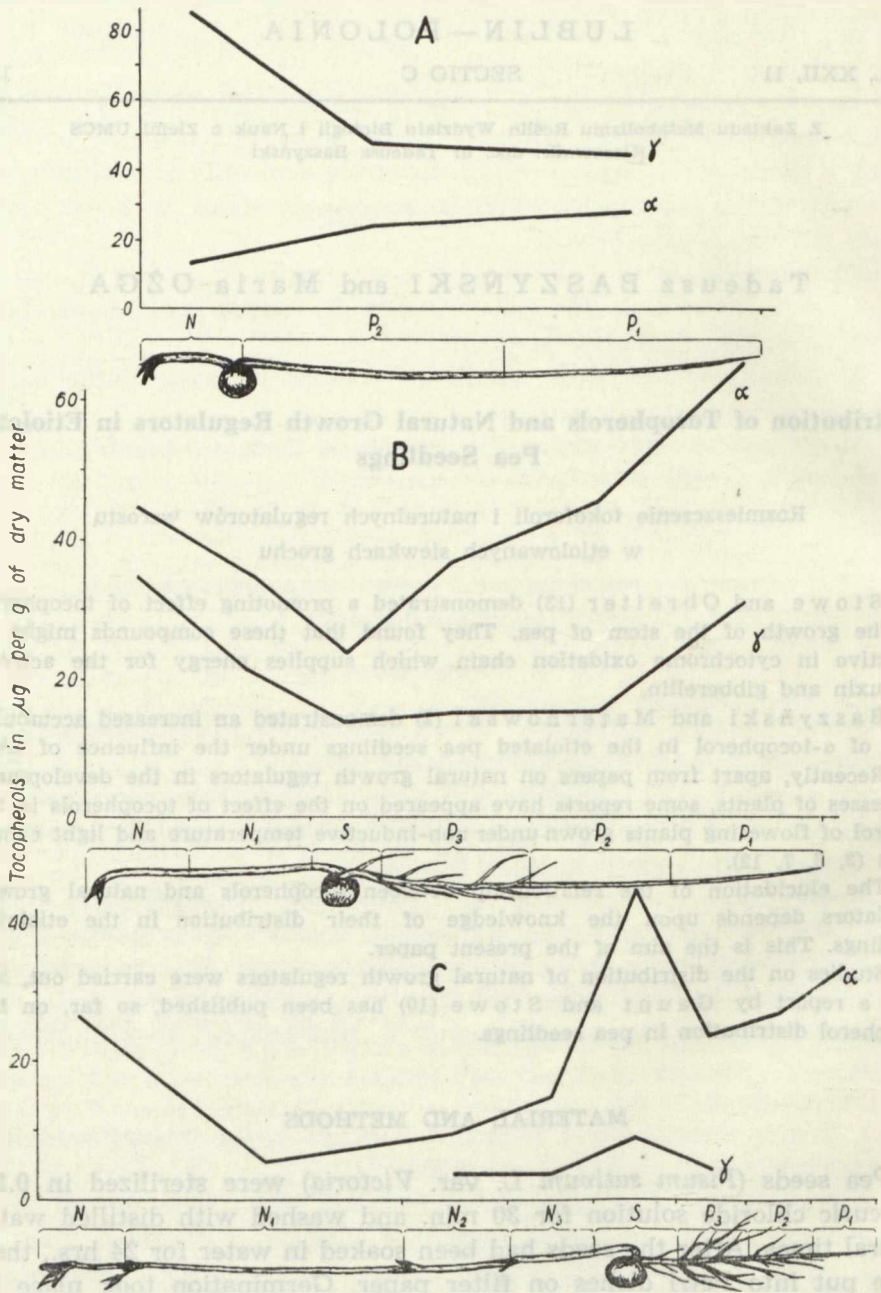


Fig. 1. The distribution of tocopherols in the etiolated pea seedlings: A — 3-day-old seedlings, 5 × (actual size), B — 5-day-old seedlings, 1.5 × (actual size), C — 10-day-old seedlings, 0.4 × (actual size)

Tocopherols were determined by the method of Green et al., accepted by The Analytical Methods Committee (1).

In order to determine natural growth regulators, 30 g of pea sections, frozen at -20°C , were extracted three times with 80% methanol. The extract was mixed and methanol was evaporated in the Rinco apparatus. The water residue was acidified with 0.1 N HCl to pH 3.5, and extracted five times with diethyl ether. Both the ether and aqueous fraction were allowed to evaporate. The ether fraction which contained growth stimulators was dissolved in 1 ml. of methanol. The aqueous fraction with inhibitors was dissolved in 1 ml. of water. The growth regulators were separated on Whatman paper No 3 in isopropanol-ammonia-water solvent (10 : 1 : 1 v/v). Separate spots on the chromatograms were eluted for 6 hrs. in 2 ml. of sucrose. The activity of the eluates was estimated by Bonner's Avena coleoptile section test. As test material for determination of stimulators and inhibitors Victory oats-Svalöf and wheat of the Opol-ska variety were used, respectively. The experiments were repeated three times. The elongation of the coleoptile sections was expressed as a percentage in relation to that of controls.

Significant differences were found by L. S. D. at $P = 0.01$.

RESULTS

Tocopherols in pea seeds are known to pass into the germ during germination. With the growth of the etiolated seedlings the relation of α - and γ -tocopherol changed in favour of α -tocopherol. In our material δ -tocopherol was not observed as its minute quantities disappeared at the very beginning of germination (2).

Table 1. The tocopherol content in the pea germ (1-day-old)

Tocopherols in μg per g of dry weight		
α	γ	Total
6.0	53.3	59.3
± 0.57	± 0.70	

The examination of tocopherols in the etiolated seedlings showed that γ -tocopherol gradually disappeared, and in the 10-day-old seedlings it was limited to that part of the plant which was in direct vicinity with cotyledons. High contents of α -tocopherol, and in younger seedlings of γ -tocopherol, were found in the apex of the stem and in roots. As the distance from the apical bud towards cotyledons increased, the level of tocopherols diminished. However, in sections close to cotyledons, which are the endogenous source of tocopherols, considerable amounts of α -tocopherols were observed. This is true especially in the case of older seedlings.

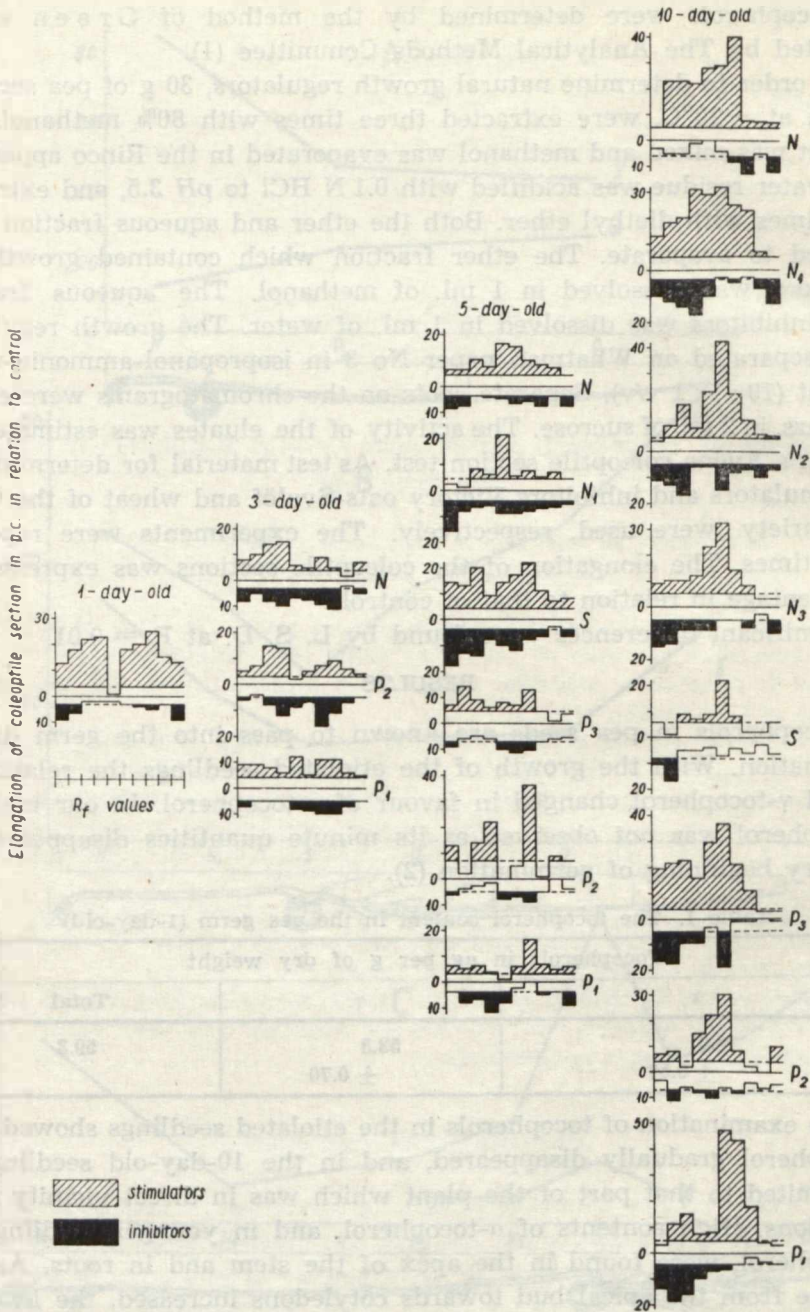


Fig. 2. The distribution of natural growth regulators in the etiolated seedlings

The tocopherol content in the etiolated seedlings was found to be in a reverse relation to the linear growth rate of the plant. The report by Booth and Hobson-Frohock (5) about α -tocopherol content in leaves being reversely proportioned to the growth rate of the plant has been confirmed in our results. A considerable number of stimulators in one-day-old seedlings slightly decreased in 3-day-old seedlings to increase again with the age of the plant. With the growth of seedlings the level of stimulators in stems was found to increase parallel to the number of stimulators found in roots. A similar relationship was also observed by Cartwright *et al.* (8).

The highest level of stimulators was found in spots with R_i ranging from 0.6 to 0.8 and corresponded to IAA.

In older seedlings higher amounts of this compound can be observed in the apical part of the plant, and these observations are in agreement with those reported by Thimann (14) on the *Avena* coleoptile. Higher amounts of IAA are in agreement with a reverse distribution of IAA oxidase in various parts of the etiolated pea epicotyl (9). Biswas (4) observed a similar distribution of kinetin-like substance in pea seedlings.

The distribution of the inhibitors was found in all the sections examined. It was uneven and the number of the inhibitors was considerably lower than that of the stimulators, particularly in the apical section of the stem and in roots. This is true especially in the case of 10-day-old seedlings.

The comparison of the results shows that the highest concentrations of tocopherols and natural growth regulators were found in those parts of the examined plants in which intensive metabolism took place. The occurrence of both compounds taken together may enable their activation as demonstrated by Stowe and Obreiter (13).

CONCLUSIONS

1. High contents of tocopherol and natural growth regulators were found in the apical section of the stem and roots of the etiolated pea seedlings.

2. Pea stem and root sections characterized by intensive linear growth contain low levels of tocopherols and natural growth regulators.

REFERENCES

1. Analytical Methods Committee. The determination of Tocopherols in Oils, Foods and Feeding Stuffs. *The Analyst*, **84**, 999, 1959.
2. Baszyński T., Maternowski T.: Influence of IAA on α -Tocopherol Synthesis in *Pisum sativum* L. during Germination and Early Stages of Growth. *Zeszyty Naukowe UMK w Toruniu, Nauki Mat.-Przyr.*, **12**, Biologia, 8, 1966.

3. Baszyński T.: The effect of Vitamin E on Flower Initiation in *Calendula officinalis* L. Grown in Short Day. *Naturwissenschaften*, **54**, 13, 1967.
4. Biswas P.: Identification of a Kinetin-like Substance from Pea Seedlings *Pisum sativum*. *Nature*, **204**, 4955, 1964.
5. Booth V. H., Hobson-Frohock A.: The α -Tocopherol Content of Leaves as Affected by Growth Rate. *J. Sci. Food Agric.*, **3**, 251, 1961.
6. Bruinsma J.: α -Tocopherol (Vitamin E) as a Plant Growth Regulator. *Chem. Weekbl.* **59**, 599, 1963.
7. Bruinsma J., Patil S. S.: The effect of 3-Indoleacetic Acid, Gibberellic Acid and Vitamin E on Flower Initiation in Unvernalized Petkus Winter Rye Plants. *Naturwissenschaften* **50**, 505, 1963.
8. Cartwright P. M., Sykes J. T., Wain R. L.: The Distribution of Natural Hormones in Germinating Seeds and Seedling Plants. In "The chemistry and mode of action of plant growth substances", ed. by Wain R. L., Wightman F., 1956.
9. Galston A. W.: Some Metabolic Consequences of the Administration of Indoleacetic Acid to Plant Cells. In "The chemistry and mode of action of plant growth substances" ed. by Wain R. L., Wightman F., London 1956. don 1956.
10. Gaunt J. K., Stowe B. B.: The Distribution and Metabolism of Vitamins E and K, Ubiquinone and Plastoquinone in Peas. *Plant Physiol.*, **39**, suppl, 25, 1964.
11. Michniewicz M., Kamieńska A.: Flower Formation Induced by Kinetin and Vitamin E Treatment in Cold-Requiring Plant (*Cichorium intybus* L.) Grown under Non-inductive Conditions. *Naturwissenschaften*, **51**, 295, 1964.
12. Michniewicz M., Kamieńska A.: Flower Formation Induced by Kinetin and Vitamin E Treatment in Long-day Plant (*Arabidopsis thaliana*) Grown in Short Day. *Naturwissenschaften*, **52**, 623, 1965.
13. Stowe B. B., Obreiter J. B.: Growth Promotion in Pea Stem Sections. II. By Natural Oils and Isoprenoid Vitamins. *Plant Physiol.*, **37**, 2, 1962.
14. Thimann K. V.: Studies on the Growth Hormone of Plants. VI. The Distribution of the Growth Substance in Plant Tissues. *J. Gen. Physiol.*, **18**, 23, 1934.

Rozmieszczenie tokoferoli i naturalnych regulatorów wzrostu w etiolowanych siewkach grochu

Streszczenie

Badano rozmieszczenie tokoferoli i naturalnych regulatorów wzrostu w 1-, 3-, 5- i 10-dniowych etiolowanych siewkach grochu (*Pisum sativum* L. var. *Victoria*), rosnących w ciemności na bibule filtracyjnej w termostacie o temp. 23°C.

Tokoferole oznaczano według metody Greena i współprac., zaaprobowanej przez AMC (1).

Naturalne regulatory wzrostu oznaczano przy pomocy owsianego testu cylindrycznego Bonnera.

Stwierdzono, iż odcinki wierzchołkowe łodygi i korzenia akumulują więcej tokoferoli i naturalnych regulatorów wzrostu niż pozostałe. Odcinki grochu, odznaczające się intensywnym wzrostem elongacyjnym, zawierają mniej badanych związków.

Размещение токоферолов и природных регуляторов роста в этиолированных сеянцах гороха

Резюме

Исследовалось размещение токоферолов и природных регуляторов роста в 1-, 3-, 5-, 10-дневных этиолированных сеянцах гороха (*Pisum sativum* L. var. *Victoria*), растущих в темноте на фильтровальной бумаге в термостате, имеющем температуру 23°C.

Токоферол определялся по методу Грина и сотр., утвержденному комитетом аналитических методов АМС (1).

Природные регуляторы роста определялись при помощи овсяного цилиндрического теста Боннера.

Установлено, что верхушечные отрезки стебля и корня в большей степени аккумулируют токоферолы и природные регуляторы роста, чем остальные. Отрезки гороха, отличающиеся интенсивным элонгационным ростом, содержат исследуемых соединений меньше.

