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Synthesis of tocopherol in cotyledonless pea seedlings

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INTRODUCTION

The chief centre of localisation of α-tocopherol in the green parts of plants are the chloroplasts (Dam et all. 1940; Booth 1963; Dilley and Crane 1963). The synthesis of tocopherol probably chiefly occurs in the chloroplasts.

The effect of light upon tocopherol synthesis has already been observed (Zakharova 1954; Green 1958; Baszyński 1959 and others) and the fact that α-tocopherol has been always found in co-occurrence with chlorophyll (Booth 1963) may indicate the connection of this process with photosynthesis. These facts do not, however, explain the presence of tocopherol in etiolated seedlings.

The tocopherols present in etiolated seedlings may derive from the cotyledons or endosperm, which contain certain quantities of these substances or their precursors.

So far it has not been known whether tocopherols accumulate in seedlings (irrespective of light conditions) when they are deprived of their endogennic sources.

It seemed possible to obtain the answer to this question by experiments in vitro with isolated embryos.

MATERIAL AND METHODS

Pea seeds were used in these investigations (*Pisum sativum* L. var. "Victoria"). The seeds were sterilized in 1% solution of mercuric chloride for 20 min. and next in 96% ethanol for 5 min and after washing six times with sterile water, the seeds were left to soak for 1,5 hour. The embryos were isolated from the seeds and placed (one per tube) on the surface of an agar medium. The basic medium was Heller's solution with sucrose (2%) and agar (0,6%) added. The cultures were kept at 20—21°C in darkness or under light (16 hours). For illuminating the plants, six 40 W lamps (type "Daylight") were used.

After 1, 3, 5, 7, 9, 11, and 13 days the plants were taken out of the test-tubes, washed and dried on filter paper. The content of tocopherols in them was determined by Green and coworker's method recommended by A. M. C. (1959), separately for shoots and roots.

As the initial quantity of tocopherol we took the amount in cotyledonless embryos directly before placing them in test-tubes.

To investigate the effect of sucrose on the synthesis of tocopherol we used a con-

centration of sucrose from 1 to 10% in a nutrient medium and limited the investigations to seven-day-old plants.

In the experiments carried out in light, the test-tubes were covered with black paper up to the height of the column of agar.

The concentration of tocopherol was expressed in μg with reference to fresh weight, dry weight and 100 roots or shoots respectively. The results are given as means of three independent experiments±standard error of the mean.

To determine the dry weight, the material investigated was dried for 12 hours at 105°C.

RESULTS

The isolated embryos of the pea contain α-, γ-, and δ-tocopherols (Table 1). The amount of tocopherols varies with the growth of the seedlings. The dynamics of their accummulation (Tables 2, 3) during the experiments is similar to that which we observe in non-isolated seedlings (Green 1958; Baszyński 1961; Baszyński and Maternowski 1965). These results differ a little from the earlier data of Chattopadhyay and Banerjee (1952) and Zakharova (1954) who found an increase in tocopherol content per unit weight of seedlings during germination.

Table 1. Tocopherols content in embryos of pea seeds

Tocopherols μg/10² embryos			
α	γ	δ	total
4,1 ±0,3	30,6 ±1,7	3,0 ±0,3	37,7

The percentage of isomers changes to the advantage of α-tocopherol, both in the roots and in the shoots. These changes occur in plants growing in light and in darkness in a different degree. Usually the seedlings growing in conditions of light at the end of the experiment contain only α-tocopherol, while some γ-tocopherol is still present in plants growing in the dark. δ-Tocopherol disappears after 5 days of the experiment.

The total tocopherol based on dry weight decreases during the development of the seedlings. This decrease is quicker in the darkness than in light. The decrease in tocopherol per unit of dry matter does not necessarily mean the inhibition of its synthesis. It is probably the expression of a quicker growth rate (an increase in weight of the plant was determined) rather than of tocopherol synthesis. It appears from the investigations of Booth and Hobson-Frohock (1961) that the tocopherol content in the leaves depends on the growth activity of the plant. The correlation between the distribution of the growth zone and the tocopherols in the seedlings also seems to prove the correctness of such a supposition (Baszyński and Ożga). The quick and total disappearance of tocopherol in early stages of germination of the pea was observed by Kartha (1964).

Table 2. Tocopherols content of cotyledonless pea seedlings based on dry weight (darkness conditions)

Day of growth	Tocopherols µg/g of dry matter						
	shoots			roots			total in whole seedlings
	α	γ	δ	α	γ	δ	
1	55,5 ±2,6	146,4 ±6,4	38,2 ±2,1	26,7 ±0,7	58,3 ±3,6	6,7 ±0,9	331,8
3	52,4 ±3,9	58,2 ±3,0	10,8 ±0,8	45,3 ±5,3	35,8 ±1,0		202,5
5	40,3 ±1,6	23,1 ±1,5	8,6 ±0,9	42,8 ±2,8	28,4 ±1,5		143,2
7	32,9 ±2,1	14,1 ±0,7		44,7 ±2,0	19,7 ±3,0		111,4
9	33,7 ±1,0	10,2 ±0,8		51,5 ±3,2	18,0 ±0,6		113,4
11	36,3 ±3,0	9,8 ±1,2		52,1 ±4,6	15,1 ±1,1		113,3
13	32,1 ±2,3	6,9 ±0,9		76,0 ±3,0	11,3 ±0,0		126,3

Table 3. Tocopherols content of cotyledonless pea seedlings based on dry weight (light conditions)

Day of growth	Tocopherols µg/g of dry matter						
	shoots			roots			total in whole seedlings
	α	γ	δ	α	γ	δ	
1	35,0 ±3,4	145,0 ±7,2	22,5 ±1,7	50,7 ±3,1	79,3 ±2,8	48,6 ±3,4	376,1
3	44,7 ±4,3	62,9 ±2,5	19,4 ±0,6	65,9 ±6,5	39,5 ±3,1	25,5 ±0,1	267,9
5	84,8 ±2,2	38,1 ±4,7	26,2 ±2,6	61,8 ±2,5	18,9 ±4,1	6,1 ±0,6	235,9
7	112,4 ±4,6	17,2 ±1,7		71,4 ±5,3	11,7 ±0,9		212,7
9	133,1 ±2,9	14,6 ±1,7		76,9 ±1,1	9,2 ±1,1		233,8
11	154,6 ±1,7			78,7 ±2,4			233,3
13	152,2 ±3,9			77,9 ±4,4			230,1

In order to establish whether the synthesis of tocopherol in isolated embryos does in fact take place, the results of the analyses were based on 100 organs investigated (Fig's 1, 2).

The differences in tocopherol synthesis in light and in darkness are considerable. They concern, however, the shoots exclusively. In the roots the quantitative relations are similar and no essential differences are observed. The total tocopherols in the shoots of seedlings growing in the dark undergo minimal changes during the experiments and the increase of α-tocopherol is equalled by the decrease in mono- and dimethyltocol. On the basis of these results, the supposition is put for-

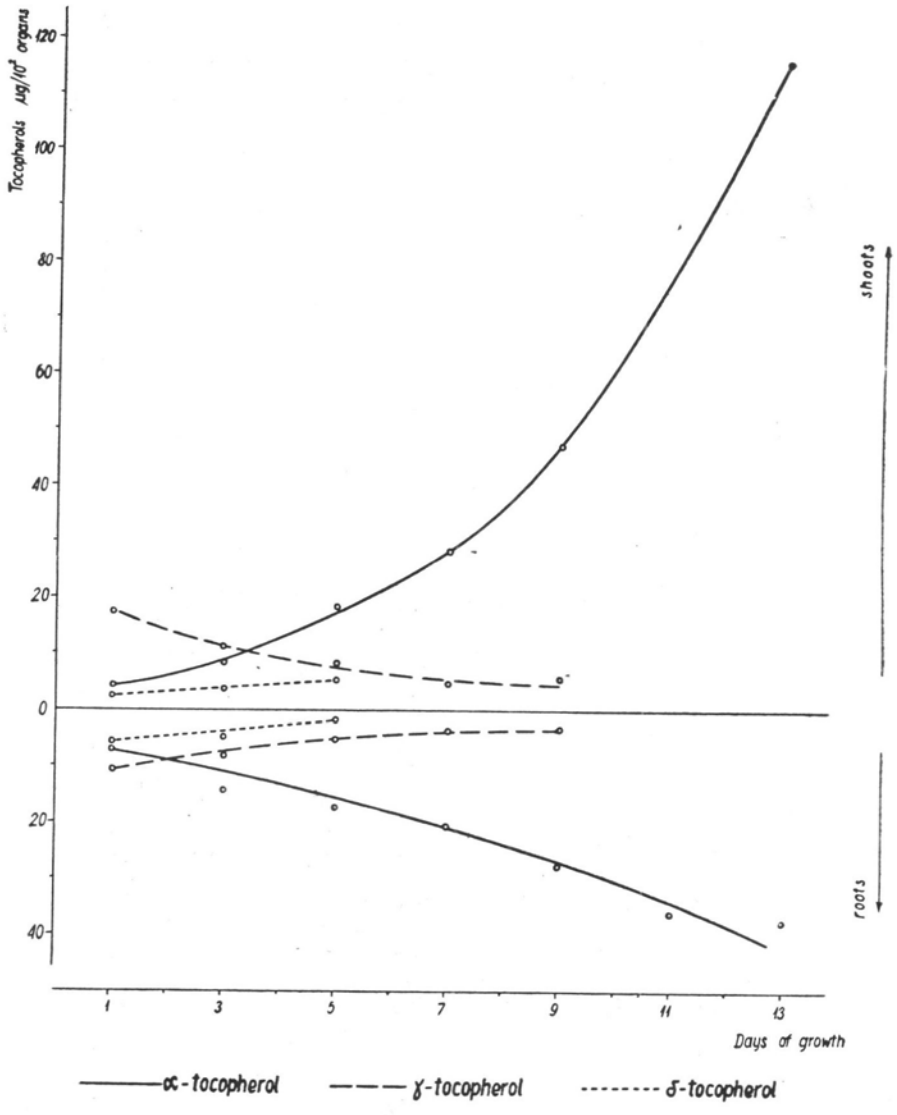

Fig. 1. Tocopherols synthesis in cotyledonless pea seedlings (light conditions)

ward that in non-isolated seedlings in darkness the accumulation of tocopherols is connected with the transport of ready tocopherols from the cotyledons, or of substrates for their synthesis.

The total tocopherols in shoots growing in light increase intensively and after 13 days their amount reaches 476% in relation to the initial amount, if we take it as 100%. Green's (1958) conclusion, that α-tocopherol is synthesized only in light, is thus confirmed.

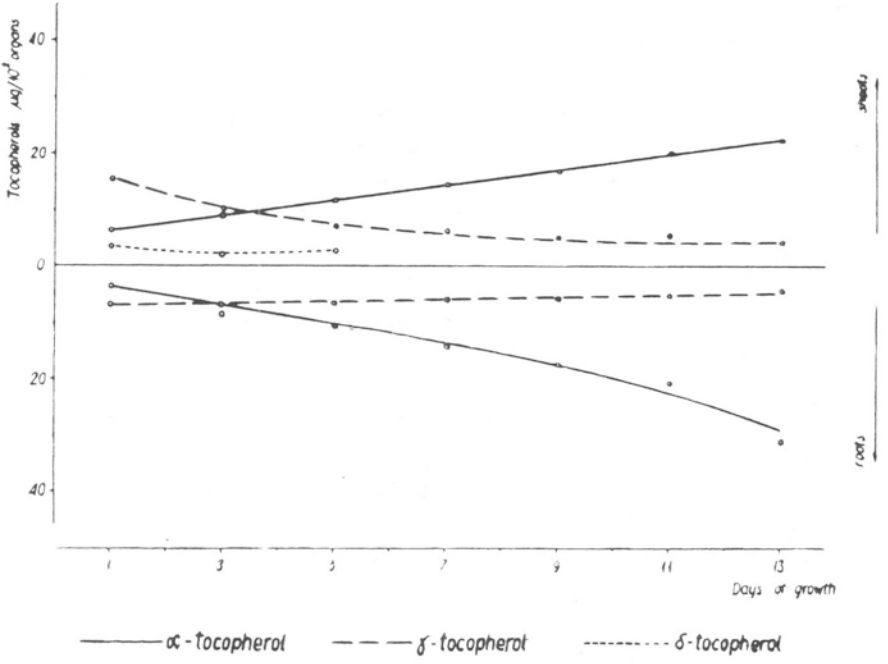

Fig. 2. Tocopherols synthesis in cotyledonless pea seedlings (darkness conditions)

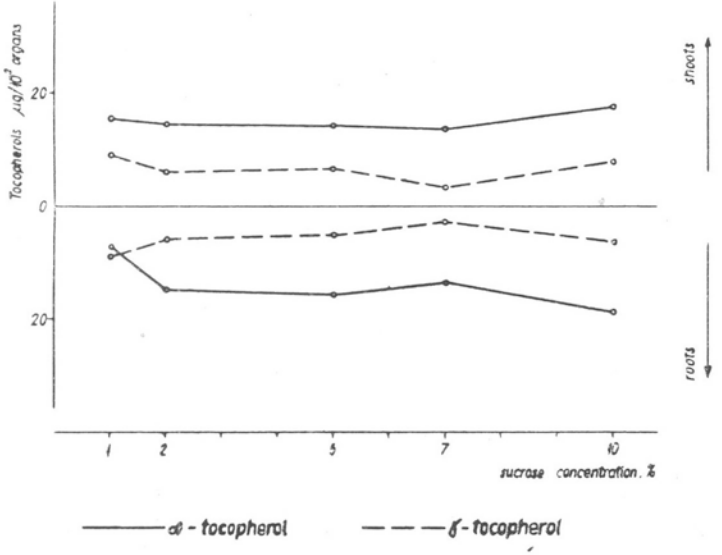

Fig. 3. Effect of external sucrose concentration on tocopherol synthesis in cotyledonless pea seedlings organs (darkness conditions)

In order to learn whether sucrose in the medium affects the level of tocopherols in etiolated organs (roots and shoots), experiments were carried out with increasing concentrations of sucrose in the medium (Fig. 3). The lack of significant differences appears to indicate that sucrose is not a substrate in the biosynthesis of tocopherols. This does not contradict the direct participation of sugars (as assimilates) in this synthesis, although Gaunt and Stowe (1964) suggest that α-tocopherol is not directly associated with photosynthesis. It is more probable, however, that this rather concerns substrates for the synthesis of the chroman nucleus. Not without significance is the role of light in the synthesis of side chains of tocopherols.

Summing up, it can be stated that in cotyledonless cultures of pea seedlings:

1. in the growing shoots – under light intensive synthesis of α-tocopherol occurs; in darkness there is no tocopherol synthesis or only in a slight degree, and the formation of α-tocopherol is connected with a change in the relation of the various isomers,

2. in the roots the course of tocopherol synthesis under the light and in darkness is similar,

3. sucrose in the medium has no significant effect on the synthesis of tocopherols.

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Summary

A substantial increase in tocopherol contents per shoot has been found in plants grown in light, while only a small rise was observed with plants kept in dark.

The tocopherol contents in roots of the two groups of plants was similar i. e. independent of light conditions.

It appears from the results that the presence of the sucrose in the medium has no effect on tocopherol synthesis.

REFERENCES

Analytical Methods Committee, 1959, The Determination of Tocopherols in Oils, Foods and Feeding Stuffs. Analyst 84, 999: 356—372.

Baszyński T., 1959, Wpływ światła na syntezę tokoferoli u *Zea mays* L. w początkowym okresie wzrostu, Acta Soc. Bot. Pol. 28 (4): 621—633.

Baszyński T., 1961, An attempt to explain the mechanism of the synthesis of α-tocopherol in the seedlings of *Pisum sativum* L., Acta Soc. Bot. Pol. 30 (2): 307—326.

Baszyński T., Maternowski T., 1965, Influence of IAA on α-tocopherol synthesis in *Pisum sativum* L. during germination and early stages of growth. Zeszyty Nauk. Uniw. M. Kopernika w Toruniu, Nauki Mat.-Przyr., Biologia (8), 12, 155—161.

Baszyński T., Ożga M., The distribution of tocopherols and growth regulators in pea seedlings (in preparation).

Booth V. H., Hobson-Frohock A., 1961, The α-tocopherol content of leaves as affected by growth rate, J. Sci. Fd Agric. 3: 251—256.

Booth V. H., 1963, α-Tocopherol, its co-occurrence with chlorophyll in chloroplasts, Phytochem. 2, 4: 421—427.

Chattopadhyay H., Banerjee S., 1952, Effect of germination on the total tocopherol content of pulses and cereals, Food Res. 17: 402—403.

Dam H., Glavind J., Nielsen N., 1940, Weitere Untersuchungen über die Bildung und Bedeutung des Vitamin K im Pflanzenorganismus, Z. physiol. Chem. Hoppe-Seyler's 265: 80.

Dilley R. A., Crane F. L., 1963, Subcellular Distribution of α-Tocopherol in Spinach and Lilac Leaf Tissue, Plant Physiol. 38, 4: 452—456.

Gaunt J. K., Stowe B. B., 1964, The distribution and metabolism of vitamin E and K, ubiquinone and plastoquinone in peas, Plant Physiol., Supp. 39, xxv.

Green J., 1958, The Distribution of Tocopherols during the Lifecycle of some Plants, J. Sci. Fd Agric. 9: 801—812.

Kartha A. R. S., 1964, A study of the mobilisation of free tocopherols in germination of seeds, J. Sci, Fd Agric. 15, 11: 759—763.

Zakharova M. P., 1954, K voprosu ob obrazovanee i lokalizatsee vitamina E w rastenee, Trudy VNIVI, V, 159—163.

Synteza tokoferoli w bezliścieniowych siewkach grochu

Streszczenie

Badano możliwość syntezy tokoferoli in vitro w bezliścieniowych siewkach grochu (*Pisum sativum* L. var. Victoria) w ciemności i na świetle.

Stwierdzono, że w pędach na świetle zachodzi intensywna synteza α-tokoferolu; w ciemności tokoferole nie są syntetyzowane, względnie w znikomym stopniu a powstawanie α-tokoferolu jest związane ze zmianą stosunku poszczególnych „izomerów".

W korzeniach przebieg syntezy tokoferoli w ciemności i na świetle jest podobny.

Sacharoza w pożywce nie wywiera istotnego wpływu na syntezę tokoferoli.