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THE EFFECT OF TEMPERATURE AND LIGHT ON THE ACCUMULATION OF HOMOSERINE IN PEA SEEDLINGS

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Abstract—The accumulation of homoserine by pea seedlings in the dark was compared at 19 and 25°. The rate of accumulation was less affected by temp. than the growth rate resulting in a higher homoserine concentration in 5-day-old seedlings grown at the lower temp. There were also differences in concentration in the root and shoot parts of the seedling. When exposed to light the rate of homoserine accumulation was greatly reduced in comparison to seedlings grown in the dark, but growth rate was also reduced, resulting again in a slightly higher homoserine concentration in the root-shoot axes of seedlings exposed to light. Other factors, such as the transpiration rate, appear to affect growth to a greater extent than they affect homoserine metabolism, producing variations in the homoserine concentration of pea seedlings. Seedlings grown in 16 hr light–8 hr dark cycles accumulated homoserine during the first week but there was a marked decrease in the content of this amino acid during the second week.

INTRODUCTION

REPORTS have been made on the accumulation or disappearance of homoserine in pea plants at various stages of growth by several workers including Virtanen and co-workers,[1] Larson and Beevers,[2] Lawrence and Grant[3] and Grobbelaar.[4] We are currently investigating pea seedling metabolism by means of tracer experiments with L-homoserine-1-^{14}C. The existing studies clearly indicate that there is a spectacular increase in the content of homoserine during the first few days following germination, but give no indication whether there is an active turnover or merely an accumulation of this amino acid. In order to study the fate of labelled homoserine it seemed desirable to find conditions of growth under which the natural homoserine content was decreasing. Such a decrease would indicate an active participation in the metabolism of the seedling. To this end experiments were designed to determine the effect of temp. and light on the homoserine content of young seedlings. The results of these experiments are reported here.

RESULTS AND DISCUSSION

Temperature Effects

Grobbelaar and Steward[5] have recently discussed the effect of temp. on the homoserine content of 77-day-old pea plants. Our study was restricted to much younger seedlings. The content of this amino acid in pea seedlings was compared in the early stages following germination. One lot was maintained at a constant temp. of 19° and the other at 25°. The seedlings grew somewhat more rapidly at 25° (Table 1).

[1] A. I. VIRTANEN, A. BERG and S. KARI, *Acta Chem. Scand.* **7**, 1423 (1953).
[2] L. A. LARSON and H. BEEVERS, *Plant Physiol.* **40**, 424 (1965).
[3] J. M. LAWRENCE and D. R. GRANT, *Plant Physiol.* **38**, 561 (1955).
[4] N. GROBBELAAR, Ph.D. Thesis, Cornell Univ. (1963).
[5] N. GROBBELAAR and F. C. STEWART, *Phytochem.* **8**, 553 (1969).

TABLE 1. GROWTH RATE* OF PEA SEEDLINGS AT 19° AND 25°

Days after planting	Length of root–shoot axis (range in mm)		Dry wt. in g/100 seedlings							
			Whole seedling		Cotyledon		Root		Shoot	
	19°	25°	19°	25°	19°	25°	19°	25°	19°	25°
1	—	—	16·4	14·6						
3	8–12	29–55	16·5	15·2						
5	42–65	70–125	15·5	15·2	14·6	13·0	0·5	1·0	0·4	1·2
6	55–90	85–175	16·2	14·9	15·0	12·3	0·6	1·2	0·6	1·5
8	90–140	115–250	14·7	14·5	12·7	10·1	0·9	1·8	1·2	2·6
12	105–250	200–350	13·7	12·6	9·8	6·6	1·5	2·4	2·5	3·7
6†	57–86		16·1		14·8		0·7		0·6	
6‡	68–155		16·1		13·4		1·1		1·6	

* Grown in the dark in moist cotton wool.
† Grown 5 days at 19° and one additional day at 25°.
‡ Grown 5 days at 25° and one additional day at 19°.

Homoserine accumulated throughout the initial 12-day period (Table 2). It began to build up slightly earlier at the higher temp. but the overall rate of accumulation in the whole seedling appeared to be quite similar at both 19° and 25° from the 5th day until the end of the experiment. As the seedling became older an increasing proportion of the total homoserine was found in the root–shoot axis, with approximately twice as much in the shoots as in the roots. The amount in the cotyledons increased until the 8th day then began to decrease. It appeared that the total amount in the shoots may have reached a plateau by the 12th day at 25°.

TABLE 2. HOMOSERINE CONTENT OF PEA SEEDLINGS AT 19° AND 25°*

Days after planting	μmoles homoserine/100 seedlings							
	Whole seedling		Cotyledon		Root		Shoot	
	19°	25°	19°	25°	19°	25°	19°	25°
1	23	8						
3	22	350						
5	1270	930	540	190	330	250	400	490
6	1580	1240	710	120	280	420	600	700
8	2370	2890	750	200	570	460	1050	2230
12	3360	3450	390	140	860	1170	2110	2140
6†	1920		810		490		630	
6‡	1990		250		360		1380	

*, †, ‡—refer to footnotes to Table 1.

The main difference attributable to the effect of temp. appeared in the cotyledons. There was approximately three times as much homoserine in the cotyledons at 19° as there was at 25°.

Additional differences due to temp. became apparent when the data were compared on a

concentration basis. From the 5th to the 12th day at 19°, the amount of homoserine per g of dry wt. remained fairly constant in both the roots and shoots. The concentration in the shoots (850–1050 μmoles/g dry wt.) was almost twice that in the roots (460–670 μmoles/g dry wt.). At 25° the data is more variable, making it impossible to come to any conclusions about trends in homoserine concentration, but in general it was considerably lower than that observed at 19° for both roots (240–490 μmoles/g dry wt.) and shoots (400–840 μmoles/g dry wt.).

Preliminary experiments had indicated that lower temp. did result in a higher homoserine concentration in the root–shoot axis. This result made it appear possible that if seedlings were transferred from a lower to a higher temp., the short time response might be a reduction in the total homoserine content. To test this hypothesis a portion of the seedlings grown at 19° for 5 days were grown for one additional day at 25° before analysis. The complementary experiment in which seedlings grown at 25° for 5 days were grown for one additional day at 19° was also performed. The results have been included at the bottoms of Tables 1 and 2. The response of 5-day-old seedlings to a change in temp. in either direction did not result in any decrease in the total homoserine content. Rather, if there was any effect at all, temp. change stimulated homoserine production in comparison to 6-day seedlings grown at constant temp.

Light Effects

The previous experiment dealt only with etiolated seedlings. Another experiment was conducted whereby seedlings grown entirely in the dark were compared to seedlings that had been subjected to alternating 12 hr dark–12 hr light cycles after the first 3 days. The temp. was maintained at 19°. The results are presented in Tables 3 and 4.

As expected, the rate of growth under alternating conditions of light and dark was much slower. The rate of homoserine accumulation was also much slower being roughly parallel to the rate of growth. However, during this stage of seedling development, there was no indication that the exposure to light could reverse the trend to accumulate this amino acid. In the root part, the data on homoserine content indicated that by the 8th day the rate of homoserine accumulation was less affected by light than the growth rate.

TABLE 3. THE EFFECT OF LIGHT ON THE GROWTH RATE* OF PEA SEEDLINGS

Days after planting	Number of light cycles†	Length of the root–shoot axis (range in mm)	Dry weight is g/100 seedlings				
			Whole seedling	Cotyledon	Root–shoot Axis	Root	Shoot
3	0	12–30	16·9	16·3	0·6		
5	2	27–51	16·2	15·2	1·0		
5	0	40–86	13·2	12·1	1·1	0·6	0·5
8	5	58–86	13·4	12·0	1·4	0·7	0·7
8	0	60–185	18·8	14·4	4·4	2·4	2·0

* Grown at 19° in sterile sand.

† After the 3rd day some of the seedlings were subjected to alternating 12-hr periods of light and dark and were harvested at the beginning of a light period.

TABLE 4. THE EFFECT OF LIGHT ON THE HOMOSERINE CONTENT* OF PEA SEEDLINGS

Days after planting	Number of light cycles†	μmoles of homoserine /100 seedlings				
		Whole seedling	Cotyledon	Root–shoot axis	Root	Shoot
3	0	180	70	110		
5	2	880	55	825		
5	0	1110	60	950	620	330
8	5	1170	170	1000	640	360
8	0	2200	190	2010	1000	1010

*, †—refer to footnotes to Table 3.

Other Effects

In the second series of experiments just described, the results for seedlings grown in the dark should be comparable with the data for seedlings grown at 19° in the earlier experiments designed to measure the effect of temp. Comparison of the appropriate data in Tables 1–4 reveals some unexpected differences. First, the increase in dry weight of the root–shoot axis was considerably faster in the second experiments, particularly in root tissue between the 5th and 8th day (e.g. Tables 1 and 3). It must be noted that although growth was in the dark at 19° in both cases, all other conditions were not identical. The first batch was grown in cotton and no part of the seedling was ever completely exposed to the atmosphere, whereas in the second series of experiments the seedlings were in sand with the shoot part emerged by the 3rd day. The total homoserine content of the whole seedlings followed a similar pattern in both series, but the distribution and concentration in the various parts were different to a considerable degree. At the 5-day stage, in the second series, the concentration in the roots was nearly twice as high (1024 μmoles *versus* 596 μmoles/g dry wt.) whereas in the cotyledons it was much lower (5 μmoles *versus* 37 μmoles/g dry wt.) than in the first series. At this age the seedlings were very nearly the same size in both cases. In the later experiment the homoserine concentration in the roots did not remain constant, but decreased by a factor of more than two between the 5th and 8th day, (1024–420 μmoles/g dry wt.), but this corresponded to a period in which the growth rate was about three times as fast as in the first experiment. Thus it would appear that the temp. effect on homoserine concentration in the seedling tissue, within the range that was studied, was a secondary effect. The rate of homoserine accumulation was not affected as much by temp. as the growth rate and, therefore, since growth was slower at the lower temp., the homoserine concentration was higher. Factors other than temp., such as the rate of transpiration, may also affect the growth rate to a greater extent than they affect homoserine biosynthesis. These factors will produce variations in homoserine concentration even at a constant temp. It also appears that temp., but other factors as well, may have an influence on the translocation of homoserine during the early stages of growth.

Evaluation of the data presented so far does not reveal growth conditions which would obviously result in the metabolic utilization of homoserine by these young seedlings. Larson and Beevers[2] have reported that the homoserine content of peas decreases during the second week after germination. An experiment was conducted in which seedlings were grown at 25° in nutrient solution and analyzed at 7, 14 and 21 days. The results are presented in Tables 5 and 6. They confirm the trend observed by the earlier workers. The magnitude of

the reduction in homoserine content between the first and second week is, indeed, quite pronounced.

TABLE 5. GROWTH RATE* OF PEA SEEDLINGS IN NUTRIENT SOLUTION†

Days after planting	Length of root–shoot axis (range in mm)	Number of leaves	Dry weight in g/100 seedlings			
			Whole seedling	Cotyledon	Root	Shoot
7	72–135	2	15·0	10·8	1·1	3·1
14	240–370	4	15·1	4·2	4·3	6·6
21	340–480	7	18·5	3·8	4·9	9·8

* Grown in sterile sand with 16 hr light–8 hr dark period and harvested at the end of a light period.
† Hoagland's solution.[6]

TABLE 6. HOMOSERINE CONTENT* OF PEA SEEDLINGS IN NUTRIENT SOLUTION†

Days after planting	μmoles/100 seedlings				μmoles/g of tissue (dry wt.)			
	Whole seedling	Cotyledon	Root	Shoot	Whole seedling	Cotyledon	Root	Shoot
7	2530	280	950	1300	170	26	900	525
14	840	30	280	530	56	8	64	80
21	970	10	200	760	53	4	40	77

*, †—refer to footnotes to Table 5.

EXPERIMENTAL

Growth and Harvesting of Seedlings

Pea seeds (*Pisum sativum* L var. Alaska) were surface sterilized by immersion in 0·5% NaOCl solution for 30 min, washed with sterile H_2O and batches of approximately 100 seeds were allowed to germinate in moist cotton-wool or sterile sand. With the sand cultures the seeds were planted 1–2 mm under the surface so that the shoots emerged very soon after germination. In cotton, there was no emergence at all before the seedlings were harvested. No mineral nutrients were provided to the seedlings except in the last experiment where they were supplied with Hoagland's nutrient solution[6] by the irrigation method (8-hr intervals). When light was used it was provided by a combination of incandescent and fluorescent lamps at a flux of 50 lx.

Seedlings were harvested at predetermined intervals and the lengths of the root–shoot axes were measured. Each batch showed considerable variation in the size of the individual seedling and one-third of the total harvested were discarded on the basis that they were either too small or too large to be truly representative for the particular stage of development. If the root–shoot axis had attained a length of about 25 mm it was dissected[7] from the cotyledon and treated as a separate sample. After attaining a length of approximately 40 mm the root–shoot axes were further dissected into root parts and shoot parts. All material was frozen in liquid N_2 immediately after dissection.

Extraction of Homoserine

In the early experiments the frozen tissue was lyophilized and the dry weight per 100 seedlings determined. Each sample was then pulverized with a mortar and pestle and the amino acids were extracted by thorough treatment with 70% (v/v) EtOH. In later experiments the fresh tissue was weighed before it was frozen. With each sample the frozen tissue was pulverized in a cold mortar and pestle and a small portion was retained for moisture analysis by the air oven method.[8] The remainder was extracted once with boiling MeOH (4 ml

[6] D. R. HOAGLAND and D. I. ARNON, *Univ. Calif. Agric. Expt. Sta. Circ.* **347** (1938).
[7] J. M. LAWRENCE, K. M. DAY and J. E. STEPHENSON, *Plant Physiol.* **34**, 668 (1959).
[8] American Association of Cereal Chemists, *Cereal Laboratory Methods*, 7th ed., Method 44–15, AACC, St. Paul, Minn. (1962).

per g fresh). The residue was further extracted twice with 80% (v/v) MeOH, and all extracts for each sample were combined.

Analysis for Homoserine

Homoserine was determined indirectly by conversion to the corresponding lactone.[9] Extracts were concentrated by evaporation at 40°. Any resulting precipitate was removed by filtration. The procedure included the preliminary removal of acidic and neutral components by chromatography on Dowex 50W × 8.[10] The amino acid fraction was evaporated to dryness at 40°, taken up in 3 N HCl and again evaporated to dryness at room temp. with the aid of an air stream. (This treatment resulted in the complete hydrolysis of any *O*-acetyl homoserine that was present. Consequently, the tabulated results also include this compound.) The residue was dissolved in pH 5·0 citrate buffer and chromatographed on a short column as described by Moore *et al.*[11] The appropriate fractions were analyzed using the modified Rosen method.[12,13] Internal standards were used as the basis for calculating all results. At least two aliquots of each sample were subjected to the complete procedure, one with and the other without an added, known amount of homoserine. The method is accurate to ±5%.

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[9] E. VOELKERT and D. R. GRANT, *Anal. Biochem.* in press (1970).
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[11] S. MOORE, D. H. SPACKMAN and W. H. STEIN, *Anal. Chem.* **30**, 1185 (1958).
[12] H. ROSEN, *Arch. Biochem. Biophys.* **67**, 10 (1957).
[13] D. R. GRANT, *Anal. Biochem.* **6**, 109 (1963).