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HOMOSERINE IN SEEDLINGS OF THE TRIBE VICIEAE OF THE LEGUMINOSAE*

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Key Word Index—*Pisum*; Leguminosae; tribe Vicieae; non-protein amino acid; homoserine.

Abstract—Young seedlings of 10 subspecies and varieties of *Pisum* all accumulated high levels of homoserine, as did also *Lathyrus latifolius* seedlings. There was less in *L. odoratus*, and little or none in *Cicer arietinum*, *Lens Culinaris*, *Vicia faba*, and *V. sativa* seedlings.

INTRODUCTION

THE VERY striking accumulation of homoserine in young seedlings of the garden pea, *Pisum sativum*, has been repeatedly observed.[1,2] While investigating the functional significance of this, we decided to compare the behavior of close relatives of the pea in this respect. Very little is known about the amino acids of germinating seeds of the Vicieae tribe other than those of the pea, although it appears probable that high homoserine concentration occurs in seedlings of some *Lathyrus*.[3,4]

RESULTS AND DISCUSSION

Roots and shoots of germinated seedlings were analyzed for homoserine. The results are given in Table 1. Homoserine levels are cited on a fresh weight basis. A large number of moisture determinations on both root and shoot tissue fell nearly always between 89 and 94%, so that the homoserine content on a dry weight basis is about an order of magnitude greater. Since the rates of germination and early seedling growth differ considerably for many of these species, chronological age and physiological age are different. Hence total seedling length and fresh weight measurements are tabulated.

Members of *Pisum* characteristically accumulate large amounts of homoserine in young seedlings. The behavior of *Lathyrus* species is less uniform. We found as much homoserine in *L. latifolius* seedlings as in some *Pisum*, but *L. odoratus* contained much less. Simola[4] reported, in a semi-quantitative way, finding much homoserine in *L. sylvestris* seedlings, small amounts in *L. niger*, and none in *L. maritimus*. Przybylska and Rymowicz[3] gave semi-quantitative data for flowering and fruiting tissues showing varying amounts in 12 *Lathyrus* species and none in 5 others. The same tissues of *Pisum sativum*[5] contain much homoserine.

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[1] VIRTANEN, A. I., BERG, A. and KARI, S. (1953) *Acta Chem. Scand.* **7**, 1423.
[2] GRANT, D. R., and VOELKERT, E. (1970) *Phytochemistry* **9**, 985.
[3] PRZYBYLSKA, J. and RYMOWICZ, T. (1965) *Gent. Polon.* **6**, 91.
[4] SIMOLA, L. K. (1968) *Acta Botan. Fenn.* 81.
[5] GOAS, G. (1966) *Compt. Rend.* **262D**, 1534.

Probably *Cicer*, *Vicia*, and *Lens* seedlings do not accumulate large amounts of homoserine. Damadoran *et al.*[6] did not note any in chick pea seedlings.

TABLE 1. HOMOSERINE CONTENT OF SEEDLINGS OF VICIEAE

Plant species or cultivar	Days after planting	Length of root-shoot axis (mm)	Fr. wt. (g per seedling)		μmol homoserine per g fr. wt.	
			Roots	Shoots	Roots	Shoots
Pisum sativum	7	12–18	0·23	0·22	60·9	65·0
cv. Alaska	10	15–21	0·60	0·61	18·0	22·2
cv. Scotch Green	7	17–19	0·20	0·20	69·9	65·8
	10	19–26	0·49	0·58		29·5
cv. First and Best	7	19–23	0·24	0·26	51·9	56·9
	10	24–30	0·49	0·59	21·2	19·9
ssp. *saccharatum*	7	10–25	0·26	0·28	59·6	52·2
	10	30–40	0·61	0·79	24·5	37·6
ssp. *hortense* (PI343983, Turkey)	7	6–9	0·40	0·33	76·2	44·6
	10	27–39	0·68	0·81	47·8	22·1
ssp. *syriacum* (PI343993, Turkey)	7	19–27	0·15	0·19	75·1	50·8
	10	32–45	0·25	0·41	47·9	27·1
ssp. *arvense* cv. Austrian Winter	7	25–28	0·15	0·18	69·4	64·2
	10	29–34	0·26	0·44	30·4	30·7
PI 358644 (Ethiopia)	7	22–29	0·36	0·32	67·0	44·0
	10	30–44	0·55	0·83	32·8	20·7
ssp. *elatius* (PI269760, England)	7	5–8	0·29	0·27	67·3	44·3
	10	24–36	0·51	0·57	47·6	23·9
P. jomardii (PI269762, England)	7	18–27	0·27	0·25	63·8	43·4
	10	28–45	0·34	0·43	41·2	22·4
Lathyrus odoratus	10	20–25	0·09	0·14	8·5	19·9
	14	21–36	0·15	0·32	19·8	12·7
L. Latifolius	15	8–20	0.035	0·043	45·4	58·6
Vicia faba	8	16–25	0·73	0·92	0·11	0·28
V. sativa	7	15–30	0·51	0·071	0·26	0·16
Cicer arietinum	8	15–25	0·80	0·27	0·27	0·30
Lens culinaris	7	21–27	0·11	0·15	0	0
	10	30–34	0·17	0·27	0	0

EXPERIMENTAL

Origin of samples. Alaska, Scotch Green, First and Best, and Austrian Winter peas, chick peas (*Cicer arietinum*) and lentils (*Lens culinaris*) were commercial samples obtained through Dr. Van E. Wilson. Seeds of sugar peas (*Pisum sativum* ssp. *saccharatum*), sweet peas (*Lathyrus odoratus*), perennial sweet peas (*L.*

[6] DAMADORAN, M., RAMASWAMY, R., VANKATESAN, T. R., MAHADEVAN, S. and RAMDAS, K. (1946) *Proc. Indian Acad. Sci.* **23B**, 86 .

latifolius), and broad beans (*Vicia faba*) were purchased from the Burpee Seed Company. Seeds of common vetch (*Vicia sativa*) were supplied by Mr. L. P. Lilley of the U.S. Soil Conservation Service. The other *Pisum* samples were obtained from the Regional Plant Introduction Station at Geneva ,N.Y., through the courtesy of Dr. S. M. Dietz of the Pullman Regional Station.

Seedling growth. The seeds were soaked in H_2O for 4 hr, disinfected in 0·001 M $HgCl_2$ for 2 min[7], rinsed in H_2O for 2 min, and germinated and grown in vermiculite at 20° for the stated periods under continuous lighting. The growth rate of some seedling species was much less uniform than others, as indicated by the spread in lengths. In order to get reasonably consistent germination of seeds of *L. latifolius*, they were given a preliminary 3 min treatment with concentrated H_2SO_4, followed by a 7 hr imbibition period and the usual disinfection.

Homoserine determination. The roots and shoots were separately frozen in liq. N_2, immediately ground to a powder in a mortar, and added to a vol. of abs. MeOH equal to 4× the fr. wt. The MeOH was heated to incipient boiling with continuous stirring, then removed from the heat, and the extraction was continued for 30 min. After centrifugation, the residue was extracted 2× more with the same vol. of 80% MeOH for 10 min each time. The combined extracts were evaporated to dryness at room temp. in a hood air stream.

Homoserine was determined on a Technicon amino acid analyzer. Its peak overlaps that of glutamine in the system for physiological fluids and tissue extracts. It is stable in neutral and basic solutions but in acidic media there is an equilibrium between the amino acid and the lactone.[8] Glutamine, on the other hand, is converted to pyrrolidone carboxylic acid in 90 min in 0·08 M phosphate buffer of pH 6·5 at 100°.[9] Tests showed that interference by glutamine was completely removed under these conditions with no loss of homoserine.

The dried residues were dissolved in 2–4 ml H_2O plus an equal vol. of 0.16 M phosphate buffer of pH 6·5. The solutions were heated on a steam bath for 90 min, then cooled, transferred to a volumetric flask, adjusted to pH 2 with 0·1 N HCl, made to volume and filtered. Appropriate amounts were at once placed on the Technicon resin cartridges, and analyzed promptly. There is a slow loss of homoserine at pH 2 in solution and on the resin (about 10% in 5 hr at room temp.), so that a standard curve is derived for the time between adjustment to pH 2 and the start of the analyzer run, using a standard homoserine solution. A correction is applied to the analyzer result based on this curve. Since homoserine emerges fairly early in the analyzer run, an abbreviated program is readily arranged whereby 0·5 N LiOH is pumped through the column soon after homoserine goes through.

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[7] LARSON, L. A. and BEEVERS, H. (1965) *Plant Physiol.* **40**, 424.
[8] ARMSTRONG, M. D. (1949) *J. Am. Chem. Soc.* **71**, 3399.
[9] HAMILTON, P. B. (1945) *J. Biol. Chem.* **158**, 375, 397.