Phytochemistry, 1972, Vol. 11, pp. 639 to 641. Pergamon Press. Printed in England.

SHORT COMMUNICATION

AMINO ACID CHANGES ASSOCIATED WITH LOW TEMPERATURE TREATMENT OF *LOLIUM PERENNE*

S. R. DRAPER

School of Agriculture, University of Aberdeen, Aberdeen, Scotland

(*Received* 1 *June* 1971, *in revised form* 3 *September* 1971)

Abstract—Exposure of *Lolium perenne* to conditions which favour the development of cold hardiness resulted in an accumulation in the leaves of proline, serine, glycine, glutamic acid and glutamine.

INTRODUCTION

THE DEVELOPMENT of cold hardiness occurs when plants are exposed to conditions of low temperature and short day-lengths.[1,2] Changes associated with cold acclimation, or hardening, include an increase in total leaf sugar[3] and the production of specific, electrophoretically distinct, protein fractions.[3–6] Increases in free amino acids have been reported with root tissue of lucerne and the soluble nitrogenous fraction of oil turnips and oil rape is known to increase during the hardening process.[7] Perennial ryegrass (*Lolium perenne* L.) is susceptible to frost under certain conditions of growth, the extent of injury depending on variety and the level of inorganic nutrients supplied.[8] In the field, severe cold damage leads to the phenomenon of 'winter-kill'.[8] The biochemistry of the cold acclimation process is thus of considerable economic significance. In the present experiments a study was made of the effect of a low temperature and short day-length regime on the free amino acid composition of leaf tissue.

RESULTS AND DISCUSSION

Plants were grown for seven weeks under greenhouse conditions and either harvested at the end of this period or transferred to a controlled environment with the temperature maintained at 2° and with a photoperiod of 8 hr, conditions favouring the development of cold hardiness. After an interval of 14 days the leaves of cold-hardened plants were taken for analysis. Ethanolic extracts were reduced to dryness and pigments were removed with light petroleum. Asparagine and glutamine were estimated by measuring the increase in their parent acids after hydrolysis with N HCl. In comparison with samples taken before acclimation, leaves from cold treated plants showed an almost threefold increase in total amino acids when results were calculated on a fresh weight basis. This increase was not due

[1] F. WIT, *Proc. 6th int. Grassld. Congr.* Pennsylvania 1607 (1952).
[2] H. M. TYSDALL, *J. Agric. Res.* **46**, 483 (1933).
[3] U. HEBER, *Planta* **52**, 144 (1958).
[4] E. D. GERLOFF, M. A. STAHMANN and D. SMITH, *Plant Physiol.* **42**, 895 (1967).
[5] E. A. COLEMAN, R. J. BULA and R. L. DAVIS, *Plant Physiol.* **41**, 1681 (1966).
[6] S. R. DRAPER and S. E. WATSON, *J. Sci. Food Agric.* In press (1971).
[7] N. HELLESTROM, *Acta Agric. Scand.* **6**, 17 (1956).
[8] H. K. BAKER and G. L. DAVID, *Agric. Lond.* **70**, 380 (1963).

TABLE 1. DRY MATTER AND TOTAL FREE AMINO ACID CONTENT OF LEAF TISSUE

	Dry matter (mg/g)	Amino acids (μm/g)
Before cold acclimation	169	7·45
After cold acclimation	149	19·9

to dehydration of the tissue as there was little change in the dry matter content (Table 1). A comparison of the percentage amino acid composition of control and cold-hardened samples showed that the period of cold acclimation was accompanied by large increases in certain amino acids, namely serine, glutamic acid, glycine, glutamine and proline (Table 2). These compounds may be divided into two groups according to their biosynthetic origins. Serine and glycine have the common precursor 3-phosphoglycerate whereas glutamic acid, glutamine and proline are synthesized from α-ketoglutarate.[9] As there is no net breakdown of protein during cold-hardening of perennial ryegrass[6] it seems likely that the observed amino acid changes result from specific alterations in amino acid anabolism.

TABLE 2. PERCENTAGE COMPOSITION OF THE FREE AMINO ACIDS OF LEAF TISSUE

	Before cold acclimation	After cold acclimation
Aspartic acid	10·5	4·6
Threonine	15·2	2·5
Serine	10·9	27·2
Glutamic acid	11·4	17·4
Proline	2·1	14·4
Glycine	0·3	1·6
Alanine	18·4	6·8
Valine	2·3	2·7
Phenylalanine	14·1	6·3
Asparagine	10·0	7·3
Glutamine	4·7	9·4

Studies with isolated chloroplasts have indicated that the membrane systems of plant cells are sensitive to the dehydration which accompanies freezing.[10] Damage of this type may be prevented with a range of cryoprotective substances.[11] As it has been shown that proline is closely involved in the mechanism of water-deficit resistance in leaves[12] it is possible that in perennial ryegrass the large increase in the concentration of this amino acid represents a process whereby resistance to damage during freezing is increased.

[9] L. FOWDEN, in *Biosynthetic Pathways in Higher Plants* (edited by J. B. PRIDHAM and T. SWAIN), p. 73, Academic Press, New York (1965).
[10] U. W. HEBER and K. A. SANTARIUS, *Plant Physiol.* **39**, 712 (1964).
[11] K. A. SANTARIUS, *Planta* **89**, 23 (1969).
[12] G. PALFI and J. JUHASZ, *Plant Soil.* **34**, 503 (1971).

EXPERIMENTAL

Plant material. Plants of perennial ryegrass (*Lolium perenne* L.) variety S23 were raised under greenhouse conditions using John Innes potting compost. Cold acclimation was carried out in a controlled environment room maintained at 2°. Artificial lighting was provided at an intensity of 6000 lx.[13]

Extraction and analysis of amino acids. Samples of leaf tissue (5 g) were disrupted in 50 ml of 80% EtOH by grinding in a glass mortar with a little acid washed sand. The slurry was filtered through paper and the residue extracted with a further 2 × 50 ml aq. EtOH. The pooled filtrates were reduced to dryness *in vacuo* at a temperature of 40° and 25 ml H_2O + 25 ml light petroleum were added to the flask. After vigorous shaking the two layers were allowed to separate. The upper layer was discarded after the aqueous phase had been frozen overnight in the deep-freeze. This procedure was repeated twice and the final pigment-free aqueous phase was reduced to dryness and the solids dissolved in 10 ml 0·1 N HCl. Amino acids were estimated by automated column chromatography using a Technicon multi-sample analyser T.S.M. 1 (Technicon Corporation, Ardsley, New York). Norleucine was used as an internal standard. The amides asparagine and glutamine co-chromatographed with the hydroxyamino acid threonine and serine. To overcome this difficulty analyses were also carried out on samples which had been refluxed with N HCl/3 hr in N_2. This treatment results in amide hydrolysis. The increases in aspartic acid and glutamic acid gave an estimate of the amounts of asparagine and glutamine which were present in the original extract. Serine and threonine were also measured in the refluxed samples. Experiments with standard solutions showed that there was a small loss of these two amino acids during the hydrolysis treatment. Recoveries were 94% and 82% for serine and threonine respectively. These figures were applied as correction factors. Amino acids present as less than 1% of the total are not recorded.

[13] F. Lorenzetti, B. F. Tyler, J. P. Cooper and E. L. Breezse, *J. Agric. Sci. Camb.* **76**, 199 (1971).

Key Word Index—Lolium perenne; Leguminosae; amino acids accumulation in leaves; low temperature effect.