BIOLOGIA PLANTARUM (PRAHA)

20 (2) : 119—128, 1978

Proline Biosynthesis in Winter Plants Due to Exposure to Low Temperatures

M. ŠTEFL, I. TRČKA and P. VRÁTNÝ

Department of Chemistry, University of Agriculture, Praha*

Abstract. The content of bound proline sharply increased in proteins of different organs of young plants of winter rape and winter wheat exposed for 72 h to temperatures from 0 to 2 °C while it decreased only in root tips of wheat plants. Free proline which at 20 °C occurs in all plant organs only in trace amounts, accumulated considerably after 72 h exposure to low temperatures in the above-ground organs and only slightly in the roots. Free proline did not accumulate during the first 24 h at 0 to 2 °C in detached leaves of winter wheat but it was incorporated into newly synthetized proteins in which proline content increased after 6 h incubation to its maximum (+11.75% in comparison to control); the content of free glutamate sharply decreased during the first 6 h of incubation and the accumulation of bound glutamate was belated in comparison to that of bound proline. Sucrose infiltrated into detached leaves of winter wheat strongly stimulated proline incorporation into proteins at low temperatures, but it did not influence glutamate incorporation. The results suggest that the main reason for the *de novo* proline biosynthesis during the first six hours of hardening of the plants is the synthesis of proteins rich in proline; free proline accumulates later predominantly in the above-ground organs as a surplus. The above-ground organs are dehydrated in the course of the hardening process approximately to the same extent both in the light and in the dark, but proline content increases much less in the dark than in the light.

The most expressive biochemical feature of plants hardened with low temperatures is the accumulation of free and bound proline (ŠTEFL and TULACH 1964, SHIOMI and HORI 1973, SHVEDSKAYA and KRUZHILIN 1973, ŠTEFL and VAŠÁKOVÁ 1978). Proteins rich in proline accumulate at the beginning of the hardening process, free proline accumulates later as a surplus (ŠTEFL and VAŠÁKOVÁ 1978); for example, free proline accumulated in the leaves of winter barley after 24 h and in radish plants after 72 h exposure to 5 °C (CHU *et al.* 1974). The content of free proline and that of proline bound in water-insoluble proteins sharply increased in the leaves of winter wheat and winter rape hardened for 72 h at 2 °C (ŠTEFL and VAŠÁKOVÁ 1978) and with respect to OSBORNE's fractions mainly in the fraction insoluble in 0.25 N NaOH (ŠTEFL 1978). Proline content in hardened plants increases mainly in cell nucleus proteins (SHVEDSKAYA and KRUZHILIN 1973) and chloroplast proteins (ŠTEFL 1978) and with respect to plant organs mainly in the proteins of the apical meristem (SHIOMI and HORI 1973); it does not increase in cell wall proteins of winter wheat plants (ŠTEFL 1978), in which hydroxyproline does not increase either (ŠTEFL and VAŠÁKOVÁ 1978). Some biochemical aspects

Received September 20, 1976; accepted August 22, 1977

* *Address:* University of Agriculture, Department of Chemistry, 160 21 Praha-Suchdol, Czechoslovakia.

of the role of free proline have already been understood: free proline at higher concentrations induces tryptophan synthase (ŠTEFL *et al.* 1973); it represses nitrate reductase (FILNER 1966); it is a cryoprotector of functioning thylakoid membranes (HEBER *et al.* 1973); it takes part in the differentiation of generative cells and organs in the course of vernalisation (SHVEDSKAYA and KRUZHILIN 1973, SHIOMI and HORI 1973), before blossoming (VALÉE 1973), mainly at the stage of microsporogenesis (PÁLFI *et al.* 1974a, b) *etc.* Accumulation of free proline is connected, according to contemporary opinion, with plant tissue dehydration (VALÉE 1973) at different stages of ontogenesis: at low temperatures (CHU *et al.* 1974, ŠTEFL *et al.* 1975), before blossoming (VALÉE 1973), during drought periods (PÁLFI *et al.* 1973, 1974a, b), on salinized soils (STEWART and LEE 1974) *etc.*

The reasons for the accumulation of bound proline have not yet been explained satisfactorily. In this paper the results of the investigations of the rate of accumulation of free and bound proline in organs of winter wheat and winter rape plants during the initial stage of hardening with low temperatures are reported.

Material and Methods

Cultivation of Experimental Plants

Frost-resistant cultivars were used in the experiments. Winter wheat plants (*Triticum aestivum* L., var. *erythrospermum*, cv. Kaštická osinatá) were precultivated in the first series of experiments to the age of 8 days (2 leaves) and winter rape plants (*Brassica napus* L., var. *arvensis* LAM., THALL., f. *biennis*, cv. Třebíčská) to the age of 10 days (1 leaf besides the cotyledons) in Knopp nutrient modified by DVOŘÁK (1958) in a controlled environment chamber at 20 ± 2 °C, 3 500 lx of white light and 16 h photoperiod. The plants were then incubated for 72 h in closed glass aquaria with an input of surrounding atmosphere, at saturated air humidity, with these experimental variants: 1) unchilled (20 °C), light; 2) unchilled, darkness; 3) chilled (0 to 2 °C), light; 4) chilled, darkness. There were three replicates with 700 plants in each experimental variant.

In the second experimental series of investigation of the rate of proline biosynthesis, winter wheat plants cv. Mironovská 808 were precultivated to the age of 12 days (2 leaves) in sand watered once a week with Hoagland nutrient solution under the conditions described for the first series of experiments. Detached leaves were then vacuum-infiltrated with 1) distilled water; 2) 0.01 M L-glutamate; 3) 0.01 M L-glutamate + 0.5 M sucrose. The solutions determined for chilled variants were prechilled to 0 °C. The plants were incubated, after washing them with distilled water, in glass vessels at saturated air humidity in these experimental variants: 1) chilled (0 °C) and 2) unchilled (20 °C) under the above light conditions. The samples for analyses were taken after 0; 0.5; 2; 6; and 24 h, from the variants with sucrose only after 24 h incubation. Zero time was the moment of infiltration of the solutions. There were three replicates in each experimental variant.

Chemical Analyses

The plants which were kept under the conditions of the described experimental variants until killing, were divided in the first series of experiments into individual organs as indicated in Figs. 1 and 2, but whole

shoots were analyzed in the second series of experiments. Dry matter was determined in the experimental material (105 °C to constant weight, 3 replicates), total nitrogen by means of microdiffusion (6 replicates, ŠTEFL 1967). For the determination of amino acids, plant material was killed by boiling in 96% ethanol and free amino acids were eluted with 75% ethanol after homogenisation, the residuum containing bound amino acids was hydrolyzed with 6N HCl (a 2000 fold surplus with respect to N material) for 40 h at 105 °C in fused glass-tubes, the filtrates were evaporated in a vacuum rotating evaporating device at 55 °C. Proline (VRÁTNÝ *et al.* 1975) and glutamate (SPACKMAN *et al.* 1958) were then determined in the residuum, six replicate determinations in each experimental variant, using a Microtechna (Czechosl.) automatic amino-acid analyzer. The results were evaluated statistically, proline determinations in the paper by VRÁTNÝ *et al.* (1975), the results of the second series of experiments in Fig. 3.

Results

The above-ground plant parts of both investigated plant species and the roots of winter wheat plants in both the light and the dark were dehydrated after a 72 h period of cold-hardening (0 to 2 °C), younger leaves and growth apices of wheat plants and above-ground parts of rape plants to the greatest extent. By contrast, a pronounced hydration of rape root tips could be observed (Fig. 1).

The content of free and bound proline changed very differently in individual plant organs after a 72 h chilling period (Fig. 2). The content of bound proline sharply increased in nitrogen compounds from the above-ground plant parts of both plant species, in green leaves and in etiolated sheaths to the greatest extent, in rape hypocotyl and in wheat growth apices to the smallest extent (Fig. 2 II-A, B), and considerably also in both root parts of rape plants (Fig. 2 II-C), but it sharply decreased in wheat root tips (Fig. 2 II A, B). The increment of bound proline decreased in the dark in comparison to the light in older leaves, in etiolated sheaths and in growth apices of wheat plants (Fig. 2 II B).

The content of free proline in plant organs changed considerably under the influence of chilling, and in the opposite direction to the changes in the content of bound proline in N-compounds from the same organs (Fig. 2 I); it was low in unchilled plants, mainly in the roots, and it decreased downwards in the plants; it sharply increased and was quantitatively redistributed in the organs of chilled plants: a most pronounced increase occurred in younger shoots, a less pronounced one in older leaves and only a slight one in the roots of both plant species. In the dark, the increments in bound and mainly in free proline content were much lower than in the light (Figs. 2 I-B, 2 II-B).

In the experiments on the rate of changes in the content of free proline and glutamate in the leaves of winter wheat plants during the first 24 h of cold-hardening, the content of free glutamate in dry matter was 0.349 before incubation and after the infiltration of exogenous glutamate 0.505% (+44%), free proline occurred only in trace amounts. The changes in the content of *de novo* synthetized glutamate and proline were controlled after 24 h cold incubation by the *de novo* synthesis of proteins rich in proline (Fig. 3A-D).

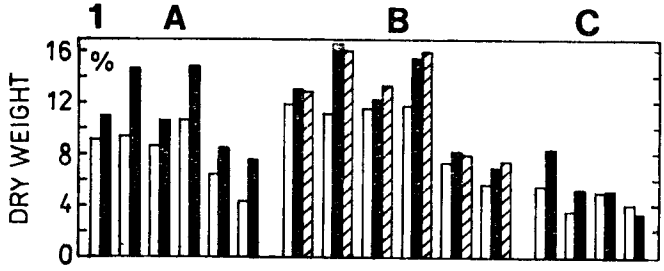

Fig. 1. Changes in dry matter content in different organs of winter wheat plants, cv. Kaštická osinatá (A, B) and winter rape plants, cv. Třebíčská (C) in the course of a 72 h cold-hardening period. The symbols are the same as in Fig. 2. Description in the text.

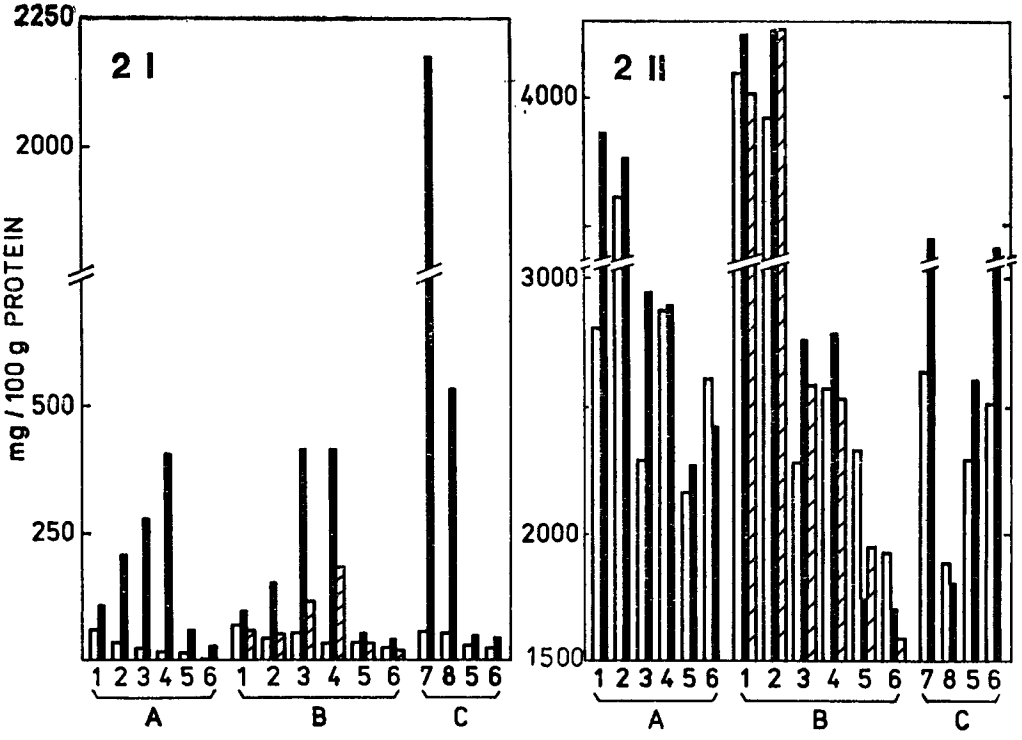

Fig. 2. The changes in the content of free (I) and bound (II) proline (mg(100 g)$^{-1}$ of all nitrogen compounds) in different organs of winter wheat plants cv. Kaštická osinatá (A, B) and winter rape plants, cv. Třebíčská (C) in the course of a 72 h cold-hardening period. 1 — the first leaf, 2 — the second (younger) leaf, 3 — etiolated leaf sheats, 4 — growth apices, 5 — roots without tips, 6 — root tips 0 to 1.5 cm + root hairs, 7 — cotyledons + the first leaf + shoot apex, 8 — hypocotyl. White area: unchilled plants, black area: chilled plants in the light, hatched area: chilled plants in the dark.

The changes in the content of both free amino acids were influenced by detachment of leaves from roots and those of glutamate (Fig. 3A) more profoundly than of proline (Fig. 3B). The content of free glutamate dropped sharply and that of free proline dropped mildly in unchilled leaves during the first 2 h of incubation, then stagnated until 6 h of incubation to rise again till 24 h. The changes of glutamate in chilled leaves were lower by approx. one third and of proline were slightly more pronounced. Sucrose

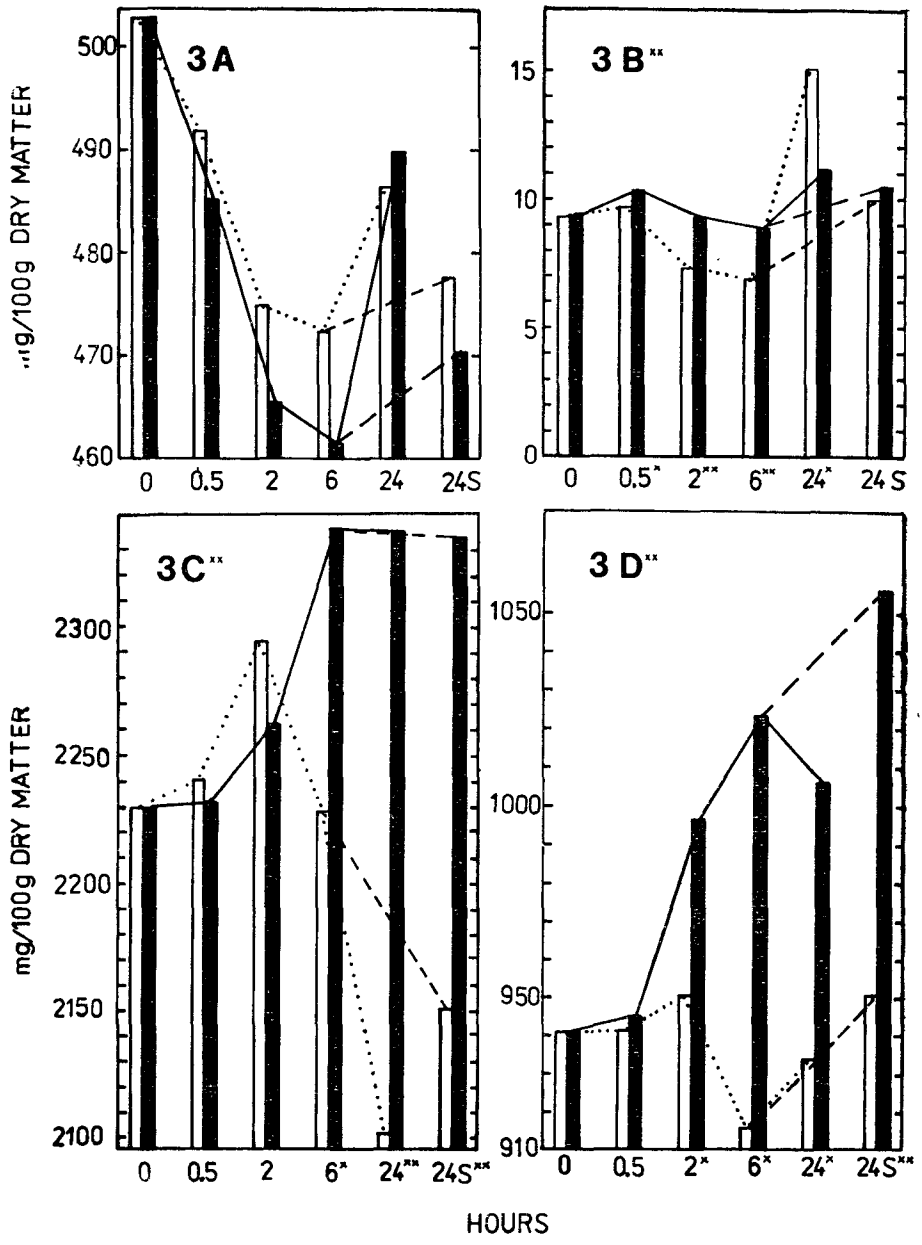

Fig. 3. The content and the dynamics of changes in the content of free glutamate (A) and free proline (B), and in bound glutamate (C) and bound proline (D) in dry matter of winter wheat leaves, cv. Mironovská 808 infiltrated with glutamate, in the period between 0 and 24 h of cold-hardening (0 °C). Shaded columns — chilled leaves (0 °C), unshaded columns — unchilled (20 °C) leaves. The columns 24S stand for the leaves infiltrated with L-glutamate and sucrose. Description in the text. The differences are statistically significant $^{x}P < 0.05$, $^{xx}P < 0.01$.

did not substantially influence the content of both free amino acids after 24 h incubation (Fig. 3 A, B, var. 24S).

The changes in the content of both bound amino acids were typical of each experimental variant. The content of bound glutamate was increased considerably (Fig. 3C) and that of bound proline slightly in unchilled detached leaves after 2 h incubation, but later glutamate content (24 h) sharply decreased and proline content (6 h) mildly decreased; after 24 h incubation the proline content rose nearly to the level of the first sampling and sucrose enhanced the increase in the content of both amino acids.

The changes in the content of both amino acids in proteins of chilled wheat leaves were controlled by a preferential, rapid biosynthesis of proteins rich in proline: bound proline content increased in 0.5 h by 3.5 mg (0.37%), in 2 h by 46.01 mg (4.8%) and in 6 h by 107.66 mg (11.75%) in comparison to unchilled leaves (Fig. 3D), thus already reaching its maximum. Bound proline content diminished between 6 and 24 h of incubation, obviously due to starvation, (+ 72.48 mg = 7.76%). Sucrose infiltrated into the leaves counteracted the decrease completely (+ 105.72 mg = 11.12%; Fig. 3D, columns 24 S). Bound glutamate also accumulated quickly, but only after the saturation of the *de novo* synthetized proteins rich in proline: its content diminished within 2 h of incubation in relation to unchilled leaves (− 32.55 mg = − 1.4%), but after 6 h of incubation its content increased to its maximum (+ 110.75 mg = 5%) and later (24 h) did not change even though in relation to unchilled leaves the absolute increment was the highest (+ 235.94 mg = 11.2%). Sucrose did not influence glutamate incorporation into proteins at low temperatures (Fig. 3C, columns 24S).

Discussion

Our results have shown that the main reason for the *de novo* synthesis of proline in organs of winter plant species in the course of the first 6 h of action of low temperatures is a preferential biosynthesis of proteins rich in proline (Figs. 2 II, 3D). The importance of these proteins has already been shown by SHVEDSKAYA and KRUZHILIN (1973) and by SHIOMI and HORI (1973). Proline content considerably increased in albumins in the leaves of winter wheat plants hardened for 60 h at 2 °C, but to the largest extent (150%) in residual proteins insoluble in 0.25 N NaOH (ŠTEFL 1978). The preferential biosynthesis of proteins rich in proline at the expense of the *de novo* synthetized glutamate (Fig. 3A, C, D) shows their specific part in plant adaptation to low temperatures. A considerable part of the proteins rich in proline accumulates in cell nuclei (SHVEDSKAYA and KRUZHILIN 1973), in the chloroplasts, but not in cell wall proteins (SHVEDSKAYA and KRUZHILIN 1973, ŠTEFL 1978). Proline incorporated into proteins is not hydroxylated to hydroxyproline (ŠTEFL and VAŠÁKOVÁ 1978), which shows that collagen-like proteins which would correspond to protective gels according to TUMANOV (1967), are not synthetized. The *de novo* biosynthesis of the proteins rich in proline sharply decreases in the dark, above all in more mature organs (Fig. 2B). What is peculiar is that bound proline does not accumulate at low temperatures in shoot apices of wheat plants and in the hypocotyl of rape plants (Fig. 2 II). The differences in the *de novo* biosynthesis of bound proline in the roots of investigated plants at 0 °C are related to specific

frost-resistance: the roots and root necks of winter rape accumulate bound proline and are considered to be a frost-resistant part of the plant (FÁBRY, personal commun.), but the roots of winter wheat plants which are supposed to be the least frost-resistant plant part (PROTSENKO and KOLOSHA 1969) do not accumulate it (Fig. 2 II).

Free proline was accumulated more in the light than in the dark in the shoots of winter wheat plants kept at low temperatures (Fig. 2 I), which is in agreement with the findings of CHU *et al.* (1974) in winter barley leaves. Free proline does not accumulate at 20 °C (Figs. 2 I, 3 A). ^{14}C-proline similarly stopped being incorporated into proteins in shoot apices of tobacco plants before blossoming at relatively low temperatures and, when exogenous proline was applied, free proline was accumulated proportionally to light intensity, inducing blossom bud formation (VALÉE 1973). It has been proved that free proline plays part in the differentiation of generative cells and organs (BRITIKOV and MUSATOVA 1973, PÁLFI *et al.* 1973, SHIOMI and HORI 1973, SHVEDSKAYA and KRUZHILIN 1973, VALÉE 1973), which agrees with accumulation of free proline in all above-ground plant organs at low temperatures (Fig. 2 I). The biochemical nature of this role of proline has not yet been understood.

The accumulation of free proline at low temperatures is remarkable from two points of view. First, the *de novo* synthetized free proline is redistributed and accumulated most in growth apices of wheat plants and in the hypocotyl of rape in which bound proline content does not rise and, on the contrary, is not accumulated in the roots. At the same time bound proline is accumulated considerably in the root tips (Fig. 2 I, II). Accumulation of free proline in the shoots and not in the roots has been reported by many authors in different plants (STEWART *et al.* 1966, SALCHEVA and GRAMATIKOVA 1967, CHU *et al.* 1974 a.o.). So far, the explanations of the biochemical part of proline are incomplete and scarce. *E.g.* HEBER *et al.* (1973) assume that free proline can act besides free sugars as a cryoprotector of functioning thylakoid membranes which, however, does not explain its part in non-chlorophyllous above-ground organs (Fig. 2 I) and, moreover, that the content of free sugars is always so high in the course of ontogenesis (ŠTEFL and TULACH 1964, 1976) that sugars could protect the membranes even without proline. At low temperatures, proline accumulation is the most conspicuous among all the other compounds (ŠTEFL and TULACH 1964, PROTSENKO and KOLOSHA 1969, ŠTEFL *et al.* 1975). In our opinion, proline plays its main regulatory role in the sphere of enzyme regulation: proline similarly as IAA which serves as an inductor of the biosynthesis of nucleic acids and proteins (KEFELI 1974), induces the biosynthesis of tryptophan synthase (ŠTEFL *et al.* 1973). Tryptophan synthase activity and tryptophan content increase abnormally before blossoming in bean leaves (ŠTEFL and SPIESSOVÁ 1970) when free proline accumulates in the plants (VALÉE 1973). Increased concentrations of IAA and proline may cooperate in the induction of biosynthesis of those RNAs and proteins which are necessary for the generative process. However proline in most cases was not a modifier of enzymic reactions (MORRIS and SYRETT 1963, HORÁK *et al.* 1973, ŠTEFL *et al.* 1973, STEWART and LEE 1974).

The second relevant problem is the change of the regulatory systems of proline biosynthesis and the changes in its incorporation into proteins at the beginning of cold-hardening of plants. According to the opinion of BAICH

(1969), proline acts in plants as an autoregulating inhibitor of the first enzyme in its biosynthesis, *i.e.* allosteric glutamate kinase, the low concentration of free proline in the shoots at 20 °C (Figs. 2 I, 3B) and in the roots at both 20 and 0 °C (Fig. 2 I) corresponding to this assumption, similarly as the inhibition of proline biosynthesis by exogenous proline in maize root tips (OAKS *et al.* 1970). Increased proline concentration in the shoots at 0 to 2 °C (Fig. 2 I) shows that the ways of proline biosynthesis change or combine, *e.g. via* arginine and ornithine (DOUGALL and FULTON 1967), or *via* glutamine synthetase (YOSHINAGA FUMIHIRO et al. 1967), or that the system of regulation of basal proline biosynthesis is changed. The question of the regulation of the sharp increase in proline content in plant proteins in the first hours of exposure to low temperatures (Fig. 3) has not yet been answered.

Another relevant question is the relationship of proline accumulation to tissue dehydration in the plants (VALÉE 1973). PÁLFÍ *et al.* (1974a, b) show that free proline is accumulated at any moment in the course of ontogenesis, when water influx into the tissues is hampered for whatever reason: either as a result of exposure to low temperatures (ŠTEFL *et al.* 1975), or as a result of blossoming onset (VALÉE 1973) and of microsporogenesis, or as a result of drought (PÁLFÍ *et al.* 1973, 1974a, b), soil salinization (STEWART and LEE 1974) *etc.* However proline is accumulated most expressively in winter and at the time of microsporogenesis. Our results show that the relationship of tissue dehydration to the accumulation of proline is valid for the aboveground organs in the light; the dehydration in the dark is the same but free proline accumulates much less (Fig. 2 IB). There are complicated relationships in the roots where no pronounced accumulation of free proline occurs at low temperatures (Fig. 2 I), but where proline is incorporated into proteins in root tips of winter rape plants (Fig. 2 II) with simultaneous hydration of the root tips (Fig. 1), while proline is not incorporated to a large extent into proteins in winter wheat root tips (Fig. 2 II), in which case the root tips dehydrate (Fig. 1). PÁLFÍ *et al.* (1974a, b) suppose that free proline plays an important part in the system of osmoregulation, because as the best soluble amino acid, proline binds much water. We however suppose on the basis of previous results (ŠTEFL *et al.* 1973) that free proline acts first of all as an enzyme regulator.

Our results confirm that sucrose enhances proline incorporation but not glutamate incorporation into proteins during the initial stage of action of low temperatures (Fig. 3CD, columns 24S). OAKS *et al.* (1970) also found that glucose addition enhanced proline incorporation into proteins but simultaneously protected free proline against oxidation (see also STEWART 1973). This also indicates the enzyme-regulating part played by free sugars in plants.

References

BAICH, A.: Proline synthesis in *Escherichia coli*. A proline-inhibitable glutamic acid kinase. — Biochim. biophys. Acta **192** : 462—467, 1969.

BRITIKOV, E. A., MUSATOVA, N. A.: [Modelling of stimulative effect of pollen during parthenogenesis in *Potentilla argentea* L. Effectiveness of proline.] In Russ. — Fiziol. Rast. **20** : 661 to 667, 1973.

CHU, T, M., ASPINALL, D., PALEG, L. G.: Stress metabolism. VI. Temperature stress and accumulation of proline in barley and radish. — Aust. J. Plant Physiol. **1** : 87—97, 1974.

DOUGALL, D. K., FULTON, M. M.: Biosynthesis of protein amino acids in plant tissue culture. III. Studies on the biosynthesis of arginine. — Plant Physiol. **42** : 387—390, 1967.

DVOŘÁK, J.: [The Influence of Changes of Inner Metabolism on Accepting of Nutrients by Living Plants.] In Czech. — CSc. Thesis. Charles Univ., Praha 1958.

FILNER, P.: Regulation of nitrate reductase in cultured tobacco cells. — Biochim. biophys. Acta **118** : 299—310, 1966.

HEBER, U., TYANKOVA, L., SANTARIUS, K. A.: Effects of freezing on biological membranes *in vivo* and *in vitro*. — Biochim. biophys. Acta **291** : 23—37, 1973.

HORÁK, V., ŠTEFL, M., TRČKA, I.: The effect of free amino acids and related compounds on the activity of plant enzymes. II. Inhibition of tryptophan synthase (4.2.1.20) from the plants of *Pisum sativum* L. by amino acids and growth substances. — Coll. Czech. chem. Commun. **38** : 3532—3538, 1973.

KEFELI, V. I.: Prirodnye Ingibitory Rosta i Fitogormony. [The Natural Growth Inhibitors and Phytohormones.] — Nauka, Moskva 1974.

MORRIS, I., SYRETT, P. J.: Cyanate inhibition of nitrate reductase. — Biochim. biophys. Acta **77** : 649—650, 1963.

OAKS, A., MITCHELL, D. J., BARNARD, R. A.: The regulation of proline biosynthesis in maize roots. — Can. J. Bot. **48** : 2249—2258, 1970.

PÁLFI, G., BITÓ, M., PÁLFI, Z.: [The free proline and water stress of plant tissues.] In Russ. — Fiziol. Rast. **20** : 233—238, 1973.

PÁLFI, G., BITÓ, M., SEBESTYÉN, R.: Rapid production of protein-forming amino acids with the aid of water stress and photosynthesis. I. Proline pathway of amino acids metabolism. — Acta biol. (Szeged) **20** : 95—106, 1974a.

PÁLFI, G., KÖVES, E., BITÏ, M., SEBESTYÉN, R.: The role of amino acids during water stress in species accumulating proline. — Fyton **32** : 121—127, 1974b.

PROTSENKO, D. F., KOLOSHA, O. I.: Fiziologiya Zimostoikosti Ozimykh Kul'tur. [The Physiology of Frost Resistance of Winter Plants Species.] — Naukova Dumka, Kiev 1969.

SALCHEVA, G., GRAMATIKOVA, H.: The influence of proline on the frost hardiness of winter wheat. — Dokl. Bolg. Akad. Nauk **20** : 1073—1076, 1967.

SHIOMI, N., HORI, S.: Proline-^{14}C metabolism in barley seedlings during vernalization. — Plant Cell Physiol. **14** : 1009—1018, 1973.

SHVEDSKAYA, Z. M., KRUZHILIN, A. S.:[Dynamics of proline during vernalization and distinction of buds of biennal plants.] In Russ. — Fiziol. Biokhim. kul't. Rast. **5** : 81—86, 1973.

SPACKMAN, D. H., STEIN, W. H.,MOORE, S.: Automatic recording apparatus for use in the chromatography of amino acids. — Analyt. Chem. **30** : 1190—1206, 1958.

ŠTEFL, M.: [An improved microdiffusion method for biochemical analysis.] In Czech. — Chem. Listy (Praha) **61** : 92—98, 1967.

ŠTEFL, M.: [The changes of distribution of proline among Osborne's protein fractions, proteins of chloroplast and cell walls in green parts of winter wheat during 1-st stage of low temperature-hardening.] In Czech. — J. Univ. Agr. Praha, Ser. A, Part 1, **1978** : 301—318, 1978.

ŠTEFL, M., HORÁK, V., TRČKA, I.: [The repression and the induction of biosynthesis of tryptophan synthase in pea plants (*Pisum sativum* L.) with L-proline and 3-indole acetic acid under light and darkness.] In Czech. — J. Univ. Agr. Praha, Ser. A, Part **2**, **1973** : 59—69, 1973.

ŠTEFL, M., SPIESSOVÁ, A.: [The activity of tryptophan synthase and tryptophan content in the leaves of pea plants during ontogenesis and their relationship to the growth and development of plants.] In Czech. — J. Univ. Agr. Praha, Ser. A Part 14, **1970** : 33—45, 1970.

ŠTEFL, M., TULACH, J.: [The content of amino acids and groups of nitrogenous substances in plants and organs of marrow-stem during feeding period and the influence of frost on the changes of amino acids and nitrogenous compounds.] In Czech. — J. Univ. Agr. Praha **1964** : 333—345, 1964.

ŠTEFL, M., TULACH, J.: [The relationship of changes of free sugars in organs of grain maize during ontogenesis to development and organogenesis of plants.] In Czech. — Mag. Proc. sci. Conf. Univ. Agr. Praha 1975, Sec. Crop Prod. Pp. 311—321. Univ. Agr., Praha 1976.

ŠTEFL, M., VAŠÁKOVÁ, L.: [The biosynthesis of proline and its distribution among fundamental nitrogenous fractions in above-ground organs of winter wheat plants at subminimal temperatures.] In Czech. — J. Univ. Agr. Praha, Ser. A, Part 1, **1978** : 35—48, 1978.

ŠTEFL, M., ZEMANOVÁ, M., FÁBRY, A.: [Free amino acids in the organs of winter rape (*Brassica napus* L., var. *arvensis* (LAM.), TELL., f. *biennis*) in the autumn-winter-spring period, with the use of the retarder (2-chlorethyl)-trimethylammonium chloride.] In Czech. — J. Univ. Agr. Praha, Ser. A, Part 1, **1975** : 49—72, 1975.

STEWART, C. R.: The effect of wilting on proline metabolism in excised bean leaves in the dark. — Plant Physiol. **51** : 508—511, 1973.

STEWART, G. R., MORRIS, C. J., THOMPSON, J. F.: Changes in amino acid content of excised leaves during incubation. II. Role of sugar in the accumulation of proline in wilted leaves. — Plant Physiol. **41** : 1585—1590, 1966.

STEWART, G. R., LEE, J. A.: The role of proline accumulation in halophytes. — Planta **120** : 279 to 289, 1974.

TUMANOV, I. I.: [On the physiological mechanism of frost resistance of plants.] In Russ. — Fiziol. Rast. **14** : 520—539, 1967.

VALÉE, J. C.: [Effect of growth phases and temperature conditions on the content of free and bound proline in *Nicotiana tabacum* var. Xanthii.] In Russ. — Fiziol. Rast. **20** : 1109—1116, 1973.

VRÁTNÝ, P., ŠTEFL, M., TRČKA, I.: [Automatic determination of proline in plant material.] In Czech. — Chem. Listy (Praha) **69** : 379—383, 1975.

YOSHINAGA, FUMIHIRO, TAKEDA, YUSUHITO, OKAMURA, SHINJI: Glutamate kinase activity in *Brevibacterium flavum:* Relationship between L-proline and L-glutamine biosynthesis. — Biochem. biophys. Res. Commun. **27** : 143—149, 1967.

BOOK REVIEW

ARNOLD, G. W., de WIT, C. T. (ed.): **Critical Evaluation of Systems Analysis in Ecosystem Research and Management.** — Pudoc, Wageningen 1976. Pp. 109, 38 figures, 4 tables. Hfl. 20.00.

This publication has appeared as the 8th volume of Simulation monographs which is a series on computer simulation in agriculture and its supporting sciences. It is a collection of seven papers, most of which were presented at the 1st International Congress of Ecology at the Hague in September 1974.

The main object of the authors is to summarize the experience with the modelling method in the first phase of its development and to give a critical commentary to its misuse by many ecologists. In the introductory article: "Some speculation on simulation", the editors discuss several conceptions and aspects of simulation method and support the opinion that the purpose of a model is to explain — not only describe — a system. The second paper by D. W. Godall: "The hierarchical approach to model buildings" has a more instructive feature, giving information on a "modular" approach in construction of a model. Dividing a system into subsystems and a model into subroutines has many advantages, *e.g.*, with respect to the testing errors of the model outputs. The third article by M. van Keulen: "Evaluation of models" is concerned with testing the validity of a model and with possible errors met in modelling a procedure. The next paper by Donald A. Jameson: "Management of ecosystems: information supplied by simulation" contains general comments on the significance of model approach for biome research and lays emphasis on the use of models for "providing a clear identification of research priorities so that research can be more specifically directed to critical problem areas". This is one of important, though many times neglected, objects of modelling. The paper by P. C. Miller and A. Moony: "The origin and structure of American arid zone ecosystems. The producers: interactions between environment, form and function" is not of a methodological character but gives the results on modelling work concerning the ecosystem research in the Mediterranean regions of California and Chile. The paper by N. G. Seligman: "A critical appraisal of some grassland models" gives a critical review of 13 grassland models elaborated by various scientists. The author claims for scientific rigour in modelling work, which has often been replaced by pragmatic usefulness. In his pioneer paper Seligman has pointed out that the critical appraisal of models should become a permanent feature of modelling activity. The concluding article by Jeffers: "Future prospects of system analysis in ecology" reviews the present state of system analysis and suggests its future development. His prognosis is sober and realistic. Only models capable of verification have scientific value for ecological studies. The methods of verification of a model have not reached a satisfactory level up to now.

The book Critical Evaluation of System Analysis in Evosystem Research and Management deserves most attention of those who are involved in modelling the ecosystems, especially of those who are over-optimistic exponents of this method and are incapable in their enthusiasm of seeing its pitfalls.

J. P. ONDOK (*Třeboň*)