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RAPID PRODUCTION OF PROTEIN-FORMING AMINO ACIDS WITH THE AID OF WATER STRESS AND PHOTOSYNTHESIS I. THE "PROLINE PATHWAY" OF AMINO ACID METABOLISM

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Abstract

Depending on their response to water-stress, it is possible to classify plants in two groups: (1) types accumulating free proline, and (2) those not accumulating proline. The differentiation can be made on flowering (microsporogenesis) by the method of the rapid "live-wilting" of the isolated leaves and shoots. Proline accumulation in the leaves in the event of water-deficit is advantageous, for it significantly increases the amount of strongly bound water, and of the protein-forming amino acids proline has by far the highest water-solubility and can therefore remain in an active state.

On oxidative, acidic hydrolysis, all of the protein-forming amino acids decomposed with the exception of proline, i.e. it is very stable. When proline is formed from glutamic acid, the reducing energy resulting from the photosynthesis is stored, and after the cessation of the water-deficit is released again when glutamic acid is re-formed. Proline compensates the 2,4-DNP-induced respiration-inhibition, and of the amino group donors (glutamate, glutamine, aspartate, asparagine, alanine, arginine, etc.) least inhibits the growth and division of the cells when in high concentration. By the "artificial live-wilting" of the isolated leaves and shoots their free amino acid content (and protein value) can be increased.

Introduction

Studies have been made of the water-deficit of field-grown plants in the entire process of development of strong water-stress, from the beginning of the water-deficiency (PÁLFI, 1968a, b; PÁLFI and BITÓ, 1970; PÁLFI et al., 1973; PÁLFI and JUHÁSZ, 1969, 1971). The contents of water, dry matter, carbohydrate, soluble total protein and free protein-forming amino acids were investigated in leaf samples taken daily. The results were controlled with a regulated water-supply in breeding vessels. The reverse process was also carried out: detailed analyses were performed after the water-supply was once established at the optimum level by irrigation at the time of strong soil-dryness.

Individuals of 80 cultivated plant species belonging to 14 families were examined. It was found that at the time of "development" of the water-deficiency the synthesis of free amino acids does not decrease, and indeed the formation of amides and some protein-forming amino acids even increases. However, proteins are not formed from free amino acids, or only as much protein is synthetized as decomposes. For this reason the plasm accumulation and the cell division come to a stop, while the most characteristic vital activities of the plants, the flow (internal movement) of the dissolved substances and the growth, decrease and then cease (ACEVEDO et al., 1971; BRITIKOV and LINSKENS, 1970; SAVITSKAYA, 1967). It is known that, even up to a 60—70% water-deficit of the leaves, the photosynthesis does function, but to a decreased extent (ACEVEDO et al., 1971; COWAN and TROUGHTON, 1971; REDSHAW and MEIDNER, 1972; SANTARIUS and ERNST, 1967). Since the synthesis of starch, proteins and nucleic acids (BOURQUE and NAYLOR, 1971; DOVE, 1967) stagnate, of the products of photosynthesis, besides the major amino acids, mainly the amides, the essential amino acids, and particularly proline, are stored to greater extents up to the exhaustion of the nitrogen reserves (PÁLFI, 1971b; PÁLFI and BITÓ, 1970; PÁLFI and JUHÁSZ, 1971). At such time the total protein-forming amino acid content of the leaves exceeds even 10% of the dry matter.

In the course of the analysis of the plants organ by organ it was discovered, as has also been found by others (BARNETT and NAYLOR, 1966; PÁLFI, 1968a; PÁLFI and JUHÁSZ, 1969; STEWART et al., 1966), that the amino acids accumulate only in the parts containing chlorophyll, and not in the roots, no matter how severe the water-deficiency. In the case of a water-deficit, therefore, a part is also played in the synthesis of amino acids by the assimilation of carbon dioxide. Thus, if intact plants grown under normal conditions in breeding vessels filled with soil are kept in the dark for several days, with simultaneous withdrawal of water, the protein and free amino acid contents of the leaves decrease rapidly, and proline does not accumulate either (PÁLFI, 1968a, b; PÁLFI and BITÓ, 1970).

The extensive accumulation of the free amino acids of the leaves is a particular mechanism of defence against the drying-out. With their active groups, the amino acids bind a large number of water molecules, whereby further water-loss is decreased. It is also favourable for the water-deficient plant that of all the protein-forming amino acids proline is the most highly soluble in water. It is interesting that in the event of a water-deficiency proline is formed from glutamic acid (BARNETT and NAYLOR, 1966; PÁLFI and BITÓ, 1970; PÁLFI and JUHÁSZ, 1969; STEWART et al., 1966), but its solubility in water is fifty times higher than that of glutamic acid. When the cells lose water, therefore, it is not precipitated to become inaccessible.

Result and Discussion

In the course of the experiments it was found that if the protein-forming amino acids are subjected to hydrochloric acid hydrolysis, in the presence of nitrate or some other oxidant, then all of the amino acids with the exception of proline decompose within 24 hours. In this respect, therefore, proline is the most stable amino acid. This has been reported previously too (EPPENDORFER and RILLE, 1973).

With oat-coleoptile and pea-segment tests, and with wheat and *Sinapis alba* germination experiments, the high concentration of proline ($3{,}5\times10^{-2}$ M), compared to those of glutamic acid and glutamine (which frequently appear as reserve), asparagine, alanine, and arginine, has the lowest inhibiting effect on the growth and division of the cells (PÁLFI et al., 1973). Indeed, in the event of injurious effects the free proline stimulates the normal respiration of the cells (BRITIKOV and LINSKENS, 1970; PÁLFI, 1971b). The formation of proline from glutamic acid is a several-step process requiring energy and ATP. In the case of a water.deficit, proline did not form in the leaves if a preliminary spraying was carried out with 2,4-DNP (10^{-3} M), since the oxidative phosphorylation and the ATP synthesis were inhibited (PÁLFI, 1971b).

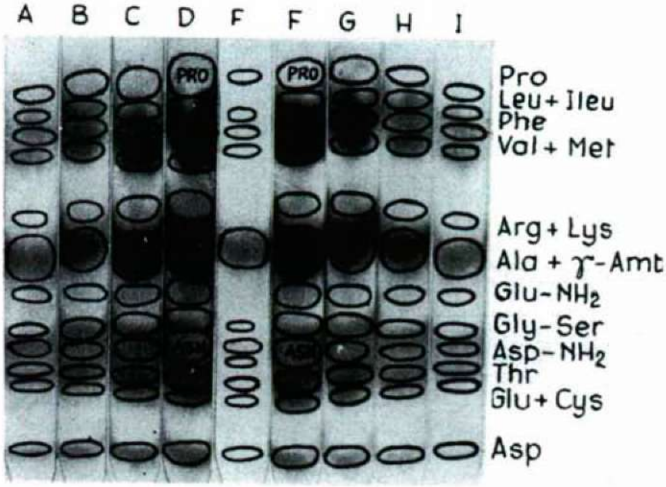

Fig. 1. Free amino acids of shoots of pea plants ("Express" variety) grown in soil-filled breeding vessels. On the effect of an increasing water-deficiency the amino acid content of the shoots increases to several times that of the control (strip E), supplied continuously with the optimum water.

A, B, C, D = 1, 2, 3 and 5 days, respectively, after cessation of irrigation. In soil suddenly saturated to the optimum water content by re-irrigation, the turgor of the shoots is re-established within a few hours, but the extremely high amount of accumulated free amino acids decreases to the normal level only after several days. F, G, H, I = 1, 3, 5 and 7 days, respectively, after re-irrigation. (The extracts refer to identical weights of dry matter.) The solvent of the paper chromatogram was phenol-water (4:1), and the developer was ninhydrine.

After the cessation of the water-deficiency the proline is reconverted to glutamic acid, and the incorporated energy is released (BRITIKOV and LINSKENS, 1970; PÁLFI, 1971b, 1972; PÁLFI et al., 1972; STEWART, 1972). This reducing energy is employed by the cells in their commencing building processes. In addition, proline is an important consitituent of the proteins of the growing cell wall, but after incorporation is converted to hydroxyproline (ROBERTS and NORTHEOTE, 1972; SAVITSKAYA, 1967). Then again the proline not only reserves energy in the critical period, but, together with the other amino acids, is the most important "protein-forming raw material" of the commencing growth processes.

The results of tissue-culture nutrient-medium experiments indicate that the proline also plays a role in the induction of the flowering (BOUNIOLS and MARGARA, 1971). A significant amount of proline can be found in the pollen too, and controls its fertility (BRITIKOV et al., 1965; SAVITSKAYA, 1967).

After the rapid saturation of the dry soil of the wilted plants with water, although the turgor of the leaves is reestablished within a few hours, the high amino acid level, and particularly that of proline, is normalized only slowly, within 6—7 days.

It turned out that not all plants accumulate proline to an extremely high extent in the case of a water-deficit. For example spinach, sugar-beet, fodder-beet, sorrel, lettuce and maize proved not to be "proline-type" plants (PÁLFI et al., 1973).

The amino acid metabolism can be considered as of the "proline-type" if, as a result of a severe water-deficiency in the leaves, the proline increases to above

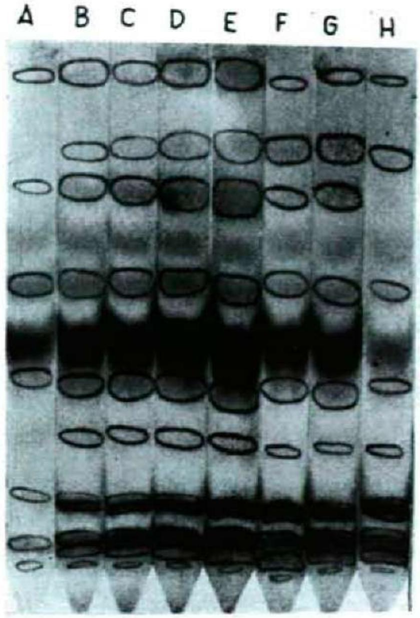

Fig. 2. Free amino acid changes in the leaves of breeding-vessel paprika plants. On the effect of a water-deficit of some days the amino acid content of the leaves increases to several times that of the irrigated control. This is shown particularly well by the proline spots (the largest and darkest spots).
A, B, C, D, E = extracts of leaves of plant not irrigated for 1, 2, 3, 4 and 5 days. After re-irrigation, the turgor of the leaves is re-established within a few hours ,but the "proline-type" amino acid accumulation decreases to the normal level only after several days. F, G, H = = 2, 4 and 8 days after saturation of the soil with water to the optimum level. All samples refer to the same weight of dry matter. The solvent was n-butanol-acetic acid-water (3 : 1 : 1), and the developer was isatine.

1% of the dry matter. Other characteristics of the "proline-type" are relatively lower glutamic acid, glutamine, γ-aminobutyric acid and arginine accumulations and, apart from the Leguminosae family, the low asparagine level too. (This definition is arbitrary, for there are also "transitional species", e.g. bean.)

It has been established that 60—70% of the soft-stemmed (herbaceous) cultivated plants are of the proline-type. The entire Solanaceae family and most genera of the Leguminosae, Cruciferae, Compositae and Graminae families exhibit proline metabolism. Although the non-proline-types are characterized by a lower total amino acid level, that of the essential amino acids is higher. The water-deficit amino acid composition shows that the "non-proline-type" plants belong mainly to species of the Chenopodiaceae and Polygonaceae families. If it is desired to study the change in metabolism induced by the water-stress, it is first worthwhile to decide whether the plant examined is of the proline-type or not. This is particularly so if the pathways and enzymes of the amino acid and proline changes are investigated.

A simple and fast method has been developed for the demonstration of the proline-type (PÁLFI et al., 1973; PÁLFI and JUHÁSZ, 1971). It has also been proved that proline accumulates to an extreme extent in the leaves only as a result of water-

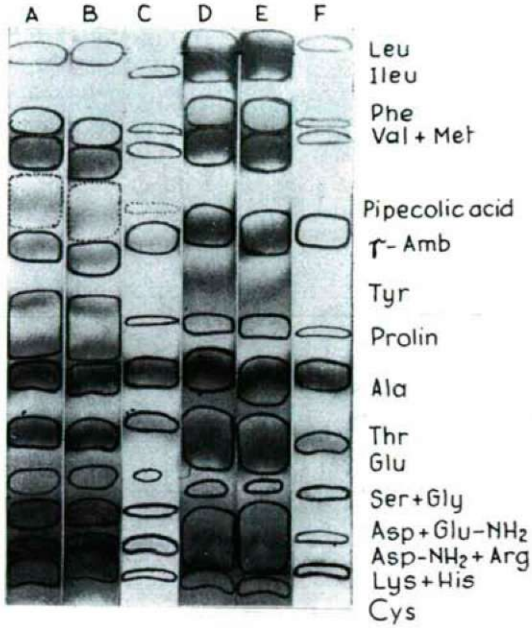

Fig. 3. The considerable increase of the free amino acid content accompanying "live-wilting" of the excised shoots and isolated leaves, with constant illumination for 2—3 days.
A, B=amino acids of "live-wilted" lucerne shoots. C=amino acids of lucerne shoots fixed immediately (not wilted) on isolation (control). D, E=amino acids of isolated "live-wilted" spinach leaves. F=amino acids of spinach leaves fixed immediately (not wilted) on isolation (control.) Every sample refers to the same weight of dry matter.
On the effect of the live-wilting the amino acid content increased to 3—4 times that of the control. In lucerne the proline was enriched to 50 times that of the control. The solvent was n-butanol-acetic acid-water, and the developer ninhydrine.

deficiency; this phenomenon can be observed in all the developmental phases of the plants (PÁLFI, 1968a, b; PÁLFI et al., 1972), but best of all at the time of microsporogenesis (PÁLFI, 1971b; PÁLFI et al., 1972).

The amino acid accumulation of the leaves and shoots attains the highest value, 5—12% of the dry matter of the leaves, in the course of a soil drought lasting for 3—6 weeks. In the leaves of plants provided with the optimum supply of water, on the other hand, the free amino acid comprises only 1,5—1,8% of the dry matter. Thus, if the control is taken as 100, then the accumulation is 300—600%.

A surprising turn of events: the "live-wilting" of the isolated leaves

It later emerged that in the course of the wilting of the isolated leaves, due to the water-loss, similar biochemical transformations take place as in the leaves of the intact plants as a result of a "severe water-deficiency". Nevertheless, there is a large difference between the two processes: during the development of their water-deficit, the leaves of the intact plants at first lose little water, and their water-deficiency "jumps" considerably only after the attainment of a very severe soil-dryness.

It is the reverse in the excised leaves: their water-loss reaches the maximum on the first day of the isolation; on the second and third days it decreases rapidly, and so a certain re-establishment is attained; only death is indicated by a slight increase in water loss.

The amino acid result from the samples taken daily revealed that the isolated leaves (shoots in the case of fodder-plants) attained the highest value of the accumulation of free amino acids at the time of their rapid water-loss, i.e. within 2—3 days; the equivalent value was measured for the intact plants only during the final days of a

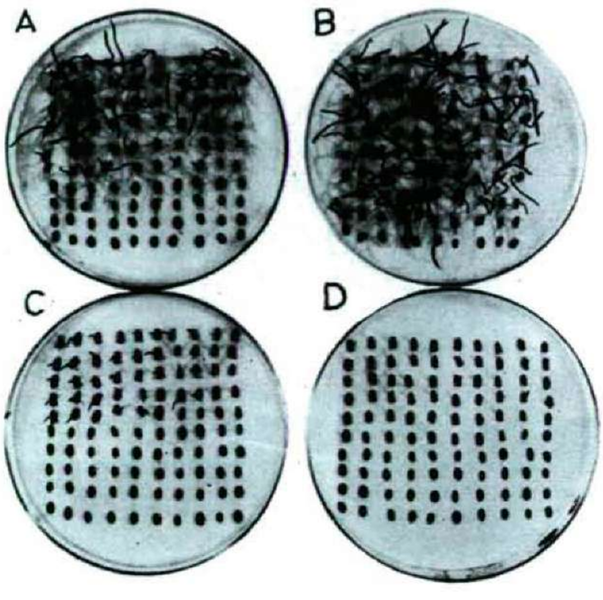

Fig. 4. The germination of wheat (variety "Bezostaya 1") in a sterile medium of a high-concentration amino acid solution ($3{,}5\times10^{-2}$ M) in tap water. Seeds germinating during 5 days are arranged in identical rows,
A = Proline solution B = Tap water (control)
C = Glutamine solution D = Asparagine solution
The proline medium inhibits the germination of the seeds less than amides storing NH_2 groups.

soil-drought lasting 3—6 weeks. In addition, with illumination for 2—3 days and under other opimum external conditions, there is no substantial change in the protein content either (COWAN and TROUGHTON, 1971; PÁLFI, 1971b; PÁLFI et al., 1972; TVORUS, 1970).

In the subsequent years attempts were made to find the optimum environmental conditions under which the protein-forming amino acid content of the isolated leaves could be increased artificially by a factor of several times. It was found that in the course of the "continuous water-loss" of the isolated leaves the level of accumulation of the free amino acids could be controlled by the variation of the following factors:

1) the temperature, CO_2 content and humidity of the air;

2) the degree of natural or artificial illumination (and the carbohydrate content of the leaves); and

3) the duration of the live-wilting.

When the amino acid enrichment of the leaves has attained the maximum, however, there is a rapid loss of amino acid and protein during the further live-wilting. Accordingly, in artificial live-wilting the water, dry matter, carbohydrate, total amino acid and proline contents of the leaves must be measured at 12-hour intervals, and (with functioning photosynthesis) the water-loss must be stopped (fixed) suddenly at the optimum degree. Simple and rapid methods were developed for the measurement of the above indicators (PÁLFI et al., 1973; PÁLFI and JUHÁSZ, 1971).

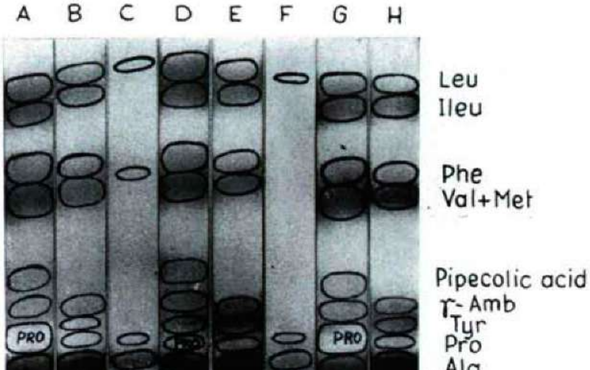

Fig. 5. Comparison of the free amino acid spectra of freshly fixed and live-wilted leaves and human tissue extracts (dividing skin tissue). With live-wilting under illumination there was a substantial increase particularly in the "essential" amino acid content of the leaves. Accordingly, the amino acid spectra of the live-wilted leaves (A, D, G) show a much greater resemblance to the amino acid composition of the human extracts (B, E, H) than do those of the freshly dried and immediately dried-out leaves (C, F). The solvent was n-butanol-acetic acid-water, and the developer ninhydrine.
A=savoy, live-wilted; B=human tissue extract; C=spinach, freshly fixed; D=spinach, live-wilted; E=human tissue extract; F=savoy, fresh; G=savoy live-wilted; H=human tissue extract.

Provoked water-deficiency and "photosynthesis operation"

The route was found for rapid biological amino acid production, and the amount thus obtained can readily be extracted by means of a simple boiling with water. With several repetitions in the cases of savoy and pea, for instance, a protein-forming total amino acid content exceeding 10% of the dry matter was attained experimentally.

It is certain, however, that other plant species and "live-wilting conditions" can be chosen, whereby even better results can be achieved. Such a method, for example, is NPK plant nutrition through the leaves on the days prior to excision, and the infiltration of the isolated leaves, or the artificial increase of the atmospheric CO_2 content and of the leaf carbohydrate content (COWAN and TROUGHTON, 1971; PÁLFI, 1971a). The presence of the carbohydrates inhibits the oxidation of proline and other amino acids (STEWART, 1972). Besides the provoked water-deficiency, therefore, there is a need for "operation of the photosynthesis" in the course of the live-wilting (COWAN and TROUGHTON, 1971). The higher CO_2 concentration of the live-wilting medium is important for two reasons:

1) it increases the intensity of photosynthesis and
2) it decreases the opening of the stomata.

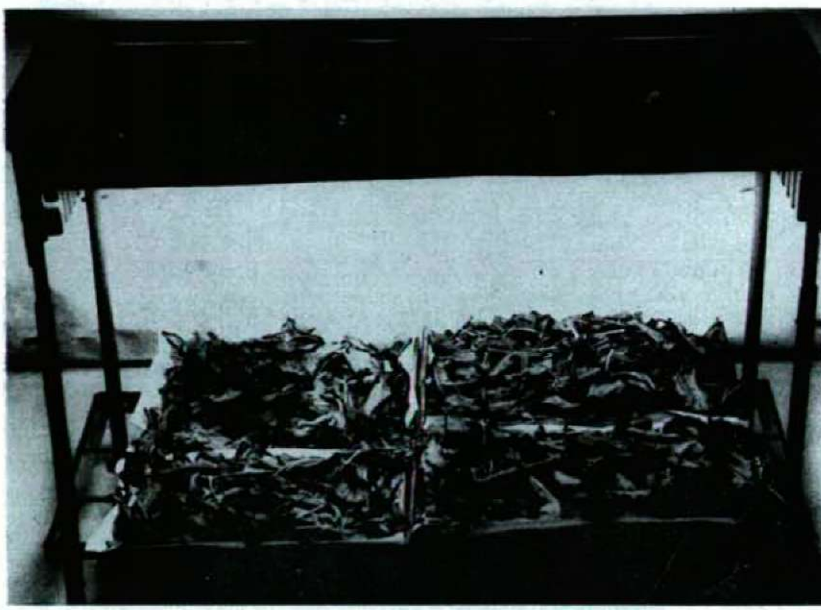

Fig. 6. Artificial increase of the amino acid content of the isolated leaves by live-wilting under laboratory conditions. The layer thickness of the spinach leaves, spread out on trays, was about 10 cm. The leaves were illuminated from above by 5 normal neon tubes at a heigh of 60 cm. In air of appropriate temperature and humidity the live-wilting combined with photosynthesis leasts 2 days. After this the material enriched in protein-forming amino acids and preserved by mild drying (dehydrated) is powdered and compacted, and packaged free from air and light.

In certain plants, with optimum light and gradual, but forceful live-wilting, the proline content of the isolated leaves can attain even 4—5% of the dry matter during 3—4 days in the course of the photosynthetic amino acid enrichment (PÁLFI, 1972). At such time, the free proline comprises 50—70% of the total amino acid. Such a plant can be regarded as a "proline works" (similarly to a "sugar works").

Table 1. Increase of free proline and total amino acid contents of isolated leaves and shoots by means of live-wilting with constant illumination. In the course of the live-wilting, with lasted 2—4 days, the total amino acid content increased 3—6 times as a percentage of the dry matter of the non-wilted, fresh control. The proline accumulation of "proline-type" plants may even increase 100 times; spinach and sorrel are not proline-type plants

PLANTS	PROLINE mg/g dry matter			TOTAL AMINO ACID mg/g dry matter		
	FRESH	LIVE-WILTED	GROWTH AS PER-CENT OF FRESH	FRESH	LIVE-WILTED	GROWTH AS PER-CENT OF FRESH
LUCERNE shoots	0,6	18,3	3 050	17,1	73,8	431
PEA shoots	0,5	21,4	4 280	20,3	124,5	613
WHEAT shoots	0,3	26,6	8 866	19,3	80,4	411
Perennial ryegrass shoots	0,4	28,1	7 025	19,2	82,4	429
SUNFLOWER leaves	0,2	23,4	11 700	12,6	41,5	329
SAVOY leaves	0,5	57,6	11 520	18,8	116,2	618
SPINACH leaves	0,4	2,2	550	16,2	68,6	423
SORREL leaves	0,3	1,7	566	15,7	47,6	303

In the repetitions of the analyses the standard deviation of the mean error is within ±8%. The live-wilting of savoy heads was performed on isolated leaves.

It is true that the sugar content of the sugar-beet is five times the proline content, but the price of proline is ten thousand times that of sugar.

It was found that 4—12 hours after the isolation the leaves have "re-adjusted" their water-loss to a certain extent. If this adaptation is disturbed by spraying with water, the decreased vital intensity (respiration, transpiration) suddenly increases. In such a case isolated leaves (spinach, sorrel and *Brassica oleracea* species) or shoots (pea, lucerne, clover and Graminae) "consume" their nutrient reserves (GENKEL' and KUSHNIRENKO, 1971; PÁLFI and JUHÁSZ, 1969).

From the aspect of the live-wilting enrichment of protein-forming amino acids, there are two essential differences between plants suitable for human nutrition (leaf-vegetables) and those used as animal fodder, as regards the processing:

1) Spinach, sorrel and *Brassica oleracea* varieties, i.e. the leaf-vegetables, must be washed several times within 3—5 hours of harvesting. During this the leaves are completely saturated with water, and the photosynthetic live-wilting begins from an "optimum physiological level". With the green foods there is no washing, i.e. no saturation with water, and thus a good result can be achieved only with plants provided with the optimum water or irrigated prior to the excision.

2) The green-vegetables generally have larger leaves, and the leaf blade too is thick and fleshy; their venation is multibranched, and so the "isolated leaves"

Table 2. Amino acid enrichment of illuminated isolated, live leaves. On the effect of live-wilting the total amount of protein-forming amino acids increased 3—5 times (in dry matter) compared to the control, which was immediately fixed and dried out on isolation. The accumulation of the essential amino acids was substantially higher (300—600%) than that of the non-essential amino acids (200—500%). Proline was an exception. (* denotes the essential amino acid) mg/g dry matter

AMINO ACIDS	SPINACH		SAVOY		SORREL	
	Fixed control	Live-wilted	Fixed control	Live-wilted	Fixed control	Live-wilted
Leucine* + Isoleucine*	0,68	7,22	0,26	4,60	0,56	5,08
Phenylalanine*	0,45	5,36	0,35	3,52	0,47	4,10
Valine* + Methionine*	0,82	7,62	0,93	5,28	0,90	7,22
Tryptophane*	0,15	0,52	0,17	0,73	0,21	0,60
γ-aminobutyric acid	1,86	5,28	2,30	4,51	1,75	3,83
Tyrosine	0,62	2,35	0,41	0,92	0,71	2,05
Proline	0,55	3,46	0,46	50,75	0,32	2,64
Alanine	2,57	6,73	2,98	5,03	2,85	5,45
Threonine*	0,47	3,46	0,85	2,68	0,54	2,92
Glutamic acid	2,90	5,58	2,37	6,10	3,07	5,16
Glycine	0,81	3,84	0,93	2,56	0,73	3,34
Serine	1,57	5,06	1,53	4,18	1,40	4,38
Aspartic acid	1,18	5,23	3,45	5,52	1,38	4,90
Glutamine	0,51	2,82	0,80	2,47	0,32	2,76
Asparagine	0,58	3,56	0,42	2,62	0,45	2,85
Arginine* + Histidine*	0,72	4,80	0,85	5,16	0,64	4,70
Lysine*	0,33	1,03	0,27	0,85	0,26	0,92
Cyst(e)ine	0,38	1,28	0,16	1,03	0,33	1,05
Total amino acid	17,15	75,20	19,49	108,51	16,89	63,95

The measurements were carried out on a "Biocal BC 200' automatic amino acid analyser. Glutamine, asparagine, proline and tryptophane were measured by separate methods.

do not lose their water content rapidly. The venation of the leaves of the green-foods, either small-leaved (clover species) or like the (Monocotyledon) Graminae, is parallel, and therefore the isolated leaves become dried out completely within one day and wilt. In the case of the green foods then the excised shoots must live-wilt. The results of our experiments show that, if it is already in the silking flowering stage, the live-wilting of maize differs substantially from these.

Preservation by mild drying

Following this, it was necessary to elaborate a preservation procedure, by means of which leaf-vegetables enriched in free amino acids retain their excellent taste and their nutriment and vitamin contents for 1—2 years. Dehydration by mild drying proved suitable (PÁLFI, 1971a, b; PÁLFI et al., 1972). Material dried to weight constancy at the required temperature is immediately powdered, and then stored protected from air and light, in a compact form. Grinding to a powder has the advantage that, compared to the coarse leaf-vegetables, more nutritive and flavouring material can dissolve up on cooking. In addition, the compacted grindings occupy little space on storage.

On the working-up of the cooking elements of the green-vegetables, it turned out that the enriched amino acid content is at the same time a concentrated flavouring material too. For this reason, our new preparations are taste and nutrient concentrates, from 8—10 grams of which, packed in foil packets, half a litre of green-vegetables can be prepared within a matter of minutes.

The new procedure is applicable to all soft-stemmed, (herbaceous) "mesophyte", Angiospermae plants in any part or place of the world.

References

ACEVEDO, E., HSIAO, T. C., HENDERSON, D. W. (1971): Immediate and subsequent growth responses of maize leaves to changes in water status. — Plant Physiol. *48*, 631—636.

BARNETT, N. M., NAYLOR, A. W. (1966): Amino acid and protein metabolism in Bermuda grass during water stress. — Plant Physiol. *41*, 1222—1230.

BOUNIOLS, A., MARGARA, J. (1971): Influence de la nature du milieu liquide ou gélosé sur la composition en acides amines des tissues de racine d'endive (*Cichorium intybus* L.) au cours de la néoformation in vitro de bourgeons végétativs ou inflorescentiels. — C. R. Acad. Sci. Paris *1273* 1104—1107.

BOURQUE, D. P., NAYLOR, A. W. (1971): Large effects of small water deficits on chlorophyll accumulation and ribonucleic acis synthesis in etiolated leaves of Jack bean *Canavalia ensiformis* L. (DC) . — Plant Physiol. *47*, 591—594.

BRITIKOV, E. A., LINSKENS, H. F. (1970): Vliyanie prolina na pogloshchenie kisloroda tkanyami rasteniy. — Fiziol. Rast. *17*, 645—654.

BRITIKOV, E. A., VLADIMIRCEVA, S. V., MUSATOVA, N. A. (1965): O prevrashchenii prolina v prorastayushchey pyl'tse i tkanyakh pestikov. — Fiziol. Rast. *12*, 953—967.

COWAN, I. R., TROUGHTON, J. H. (1971): The relative role of stomata in transpiration and assimilation. — Planta (Berl.) *97*, 325—336.

DOWE, L. D. (1967): Ribonuclease activity of stressed tomato leaflets. — Plant Physiol. *42*, 1176—1178.

EPPENDORFER, W. H., RILLE, S. W. (1973): Decomposition in presence of nitrate of amino acids especially tyrosina, during acid hydrolysis of plant material and standard amino acid solution — Plant and Soil. *38*, 215—218.

GENKEL', P. A., KUSHNIRENKO, SZ. V. (1971): Vliyanie na dikhanie list'ev rasteniy fasoli, predposevno zakalennikh k zasukhe. — Fiziol. Rast. *18*, 947—953.

PÁLFI, G. (1968a): Die Wirkung von Kinetin, 2,4-DNP und Antimetaboliten auf die Veränderungen im Aminosäurengehalt welkender Pflanzenblätter. — Planta (Berl) *78*, 196—199.

PÁLFI, G. (1968b): Changes in the amino acid content of detached wilting leaves of *Solanum laciniatum* Ait. in the light and in the dark. — Acta Agron. Acad. Sci. Hung. *17*, 381—388.

PÁLFI, G. (1971a): Eljárás szabad amir.osavakban dúsított növényi készítmény előállítására. Magyar Országos Találmányi Hivatal. Beadás ideje: 1969. szept. 26. Engedélyezés ideje: 1971. nov. 22. Lajstromszáma 160 360.

PÁLFI, G. (1971b): Multiplication of the essential amino acids during the live-wilting of leaves. — Acta Biol. Szeged. *17*, 89—103.

PÁLFI, G. (1972): Természetes aminosav gyár (Natural factory of amino acids). — Élet és tudomány (Life and Science) *47*, 2241—2245.

PÁLFI, G., BITÓ, MÁRIA (1970): Effect of the cold soil and physiological dryness on the amino acid metabolism of wheat, beans, sunflower, and paprika. — Acta Biol. Szeged. *16*, 79—91.

PÁLFI, G., BITÓ, MÁRIA, SEBESTYÉN, RITA, IHRING, E., POSEVITZ, V. (1972): Néhány levélzöldség- és takarmánynövény szabad aminosav tartalmának fokozása élve-hervasztással. (Live-wilting, a method for increasing free amino acid content of some leaf vegetables and fodders.) — Növénytermelés. (Crop Production.) *21*, 203—214.

PÁLFI, G. ,BITÓ MÁRIA, PÁLFI, ZSÓFIA (1973): Svobodiy prolin i vodniy deficit rastitel'nikh tkaney. — Fiziol. Rast. *20*, 233—238.

PÁLFI, G., JUHÁSZ, JÚLIA (1969): Zusammenhang zwischen Wassermangel salzigen oder kalten Wurzelmedium sowie Prolin-, Pipecholinsäurengehalt der Pflanzen. — Zeitsch. für Pflanzenernahrung und Bodenkunde *124*, 36—42.

PÁLFI, G., JUHÁSZ, JÚLIA (1971): The theoretical basic and practical application of a new method of selection for determining water deficiency in plants. — Plant and Soil *34*, 503—507.

PÁLFI, G., MARÓTI, I., BITÓ, MÁRIA (1974): Teoriya sosdaniya productov rastitel'nogo proiskhoshdeniya s povishennim sodershaniem svbodonikh aminokislot, zaimstvovannaya iz biologii. — Izvestiya TSHA. Biol. (Moscow) In press.

REDSHAW, A. J., MEIDNER, H. (1972): Effect of water stress on the resistance to uptake of carbon dioxide in tobacco. — J. of Exp. Botany *23*, 229—240.

ROBERTS, K., NORTHEOTE, D. H. (1972): Hydroxyproline: observations on its chemical and autoradiographical localization in plant cell wall protein. — Planta (Berl) *107*, 43—51.

SANTARIUS, K. A., RENATA, ERNST (1967): Das Verhalten von Hill-Reaction und Photophosphorysierung isolierter Chloroplasten in Abhängigkeit von Wassergehalt. I. Wasserentzug mittels konzentrierter Lösungen. — Planta (Berl) *73*, 91—108.

SAVITSKAYA, N. N. (1967): K voprosu o nakoplenii svobodnogo prolina v rasteniyakh yachmenya pri nedosztatke vodi v pochve. — Fiziol. Rast. *14*, 737—739.

STEWART, C. R. (1972): Effects of proline and carbohydrates on the metabolism of exogenous proline by excised bean leaves in the dark. — Plant Physiol. *50*, 551—555.

STEWART, C. R., MORRIS, C. J., THOMPSON, J. F. (1966): 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.

TVORUS, E. K. (1970): Vliyanie zasukhi i povishennoy temperaturi na aktivnost' ribonucleazi v rasteniyakh. — Fiziol. Rast. *17*, 787—794.

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