Acta Biologica Szeged. 25 (1—4), pp. 49—61 (1977)

EFFECTS OF BIOLOGICALLY-ACTIVE SUBSTANCES ON THE AMINO ACID METABOLISM OF ISOLATED LUCERNE SHOOTS IN THE CASE OF LIVE-WILTING

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(Received June 20, 1976)

Abstract

The essential amino acids, proline and amides accumulate to an extremely great extent in excised lucerne shoots if these are left spread out in light to lose their water progressively over two days while still alive (we have termed the phenomenon "live-wilting").

The total free amino acid content of control shoots which are dried immediately after cutting-off is very low (1.83% of the dry matter). As a consequence of live-wilting for 2 days, this content is 8.15%, i.e. an increase of 4.45 times. Our results indicate that the digestibility quotient, the biological value and the protein-utilization of green-fodder plants can be increased by around 100% with the artificial live-wilting method (by increase of the free protein-forming amino acids).

It was found that if the shoots are treated with biologically-active substances after the cutting-off, during the live-wilting in the following 2 days the total free amino acid content can be further increased considerably. Treatment with indole acetic acid (20 mg/l) and joint treatment with indoleacetic acid (20 mg/l)+abscisic acid (5 mg/l) led to total free aminoacid increases of 20% and 60%, respectively, compared to a control treated only with water.

Although treatment with abscisic acid alone did not increase the total amino acid values of the isolated shoots, it did decrease the water turnover of the shoots by 30%, by closing the stomata. This water turnover decrease was not antagonized either by the joint effect of indoleacetic acid+ +gibberellic acid+furfurylaminopurine employed with the abscisic acid.

It was found that, when carefully dried at 70 °C and granulated, the young lucerne shoots containing an elevated amino acid content as a result of live-wilting can also be utilized as a food for humans, if sufficiently spiced. This preserved food would form a sure protein basis for feeding purposes in protein-poor countries.

At present the live-wilting procedure on isolated shoots is the most easily performable and therefore the cheapest method of producing by biological means natural amino acid mixtures representing high-level protein-utilization.

Introduction

At the time of a severe water-deficiency in the case of field-grown herbaceous plants, the intensity of photosynthesis decreases, the starch, protein and nucleic acid contents do not increase, and the growth ceases. At the same time, the synthesis and accumulation of the free amino acids are enhanced (KUDREV and TYANKOVA, 1966; SANTARIUS and ERNST, 1967; PÁLFI, 1968ab, 1971; LEWITT, 1972; PERDRIZET, 1974; VALLEE, 1973; WALDREN and TEARE, 1974). As a result of the water-deficit, in the leaves there is an accumulation primarily of the essential amino acids, the amides, and particularly proline.

If the shoots are cut off and left spread out in the light to lose their water gradually during the processes of photosynthesis while the shoots are still alive, their

total amino acid content increases to a much greater extent compared with that of the intact, field-growing water-deficient plants. During this phenomenon of „live-wilting" (PÁLFI, 1969, 1971) the isolated shoots lose 70—80% of their original water content. Such a high water-deficit can be attained mainly only in culture-vessel experiments and in the isolated shoots.

The practical importance of the water and nutrient metabolism of isolated shoots s that all cut and harvested green-fodder plants of greenstuffs undergo live-wilting for a time prior to the processing or preservation, and during this time their free, protein-forming amino acid content may increase to a considerable extent, or it may decrease, depending on the changes in the environmental factors.

By means of artifical live-wilting in a conditioned medium for 2 days, it proved possible systematically to increase the protein-forming total amino acid content of the cut-off shoots of lucerne and other fodder plants from 2.0% to 6.0—10.0%, without an accompanying decrease in the protein content (PÁLFI, 1971; PÁLFI et al., 1974ab, 1975ab). It has already been proved in animal and human experiments (RIGÓ, 1976) that a natural mixture of the protein-forming free amino-acids may be equivalent to three times the amount of proteins as regards nutrition, because of the low efficiency of digestion of the proteins.

In our experiments with isolated shoots and leaves, in only 17 of 50 herbaceous plant species belonging to 14 families did it not prove possible to detect systematically a high proline content (i. e. above 1.0% of the dry matter) on the application of live-wilting. However, in the species of "non-proline type" too the total amino acid content increased to a high level. The majority of soft-stemmed plants are therefore of „proline-accumulating type" (PÁLFI et al., 1973, 1974ab). It has been shown that the level of proline accumulation is primarily a species characteristic, and is connected only secondarily with the magnitude of the water-deficiency and with the degree of drought-resistance among the cultivated varieties (PÁLFI et al., 1975a).

Physiological aridity and the accompanying high total amino acid and proline contents may be induced in the leaves by the low temperature of the culture medium, by frost, or by the elevated osmotic pressure of the medium (GOAS, 1966; HUBAC, 1967; PÁLFI, 1969, 1971; BOKAREV and IVANOVA, 1971; BATES et al., 1973; HUBER, 1974; STEWART and LEE, 1974). However, we shall not deal with this phenomenon now.

It has already been established that, during the first 6—8 hours of live-wilting, changes take place in the endogenous biologically-active substances of the shoots: the concentrations of indoleacetic acid oxidase and abscisic acid (ABA) increase rapidly, while at the same time the amount of indoleacetic acid (IAA) decreases strongly (DARBYSHIRE, 1971; ITAI and VAADIA, 1971). It follows from this that the effects of such biologically-active substances as exogenous IAA and ABA may be manifested via the magnitudes of the intensities of amino acid and protein syntheses, and also by the accumulation of these substances.

A study was earlier made (PÁLFI et al., 1975ab) of the individual effects of IAA, gibberellic acid (GA_3), furfurylaminopurine (FAP), ABA and potassium ions on the water equilibrium of isolated lucerne and lentil shoots and on the free amino acid content of the shoots during live-wilting. It was found that the considerable accumulation of the protein-forming amino acids in the cut-off fodder plants during live-wilting can be further increased substantially with certain biologically-active substances.

In the first part (A) of our present experiment, a study is made of how IAA and ABA solutions absorbed into lucerne shoots at the lower part of the stem, immediately after cutting-off, affect the water turnover and the amino acid and protein metabolisms of the live-wilting shoots. In addition, the combined effect of exogenous IAA + GA_3 + + FAP is examined, together with the question of whether the amino acid content in this variant changes if the treatment is combined with ABA.

Since it was earlier found (PÁLFI et al., 1975ab) that the level of amino acid accumulation is raised significantly by IAA solution during live-wilting, while exogenous ABA acts in decreasing the water turnover, in the second part (B) of our present experiments a study is made of the combined activities of these two growth substances, which have fairly opposite effects, and IAA and ABA treatments are also made individually.

Materials and Methods

Lucerne shoots grown in the field with the optimum water supply were divided into 100 g groups immediately after cutting-off (every variant was carried out in triplicate). In the course of this weighing procedure, the lower, aged leaves of the 25—30 cm long shoots were removed, or the lower part of the shoots was cut off. Every 100 g group consisted of 30 shoots. Next, 150 ml solutions of the active ingredients or the control water were poured into deep glass vessels, and the basal parts of the well-washed shoot groups were immersed for 24 hours in these solutions under illumination (Fig. 1). The shoots in one group (in triplicate) were cut up finely, immediately fixed and dried at 70 °C (fresh, control).

After the 24-hour illuminated absorption, the groups were reweighed in the water-saturated state. The shoot groups were then spread out, no longer in solution but on separate dishes, reilluminated, and a severe water-deficiency (water-losing live-wilting) was provoked to the accompaniment of a low atmospheric humidity, so that the shoot groups lost 70—80% of their water content during the 48-hour incubation. The shoots were next weighed once again (wilting weight). Finally, the shoot groups were chopped up, dried to weight constancy at 70 °C, their dry matter contents were weighed, and the material was then ground for purposes of extraction.

The paper and thin-layer chromatographic methods of amino acid analysis, the development being slowed by cooling, were reported previously (PÁLFI et al., 1972, 1973, 1974ab).

The proline was measured separately. Six different amounts of proline standards were also applied to the 18—22 band paper, with variants in triplicate (Fig. 2). After development and elution, the extinctions of the isatin blue solutions were measured spectrophotometrically.

The total amino acid, with the exceptions of proline and asparagine, was measured with the aid of the universal standard, an elution-colorimetric method being used (PÁLFI et al., 1973, 1974 ab). Certain analytical details were checked on an automatic amino acid analyzer (Biocal BC 200).

The soluble total protein was measured with the method of LOWRY et al. (1951). Samples with different water contents were calculated on the basis of an identical dry matter content.

Amino acid and protein analyses were carried out on 3—5 samples and the averages are reported. If the mean error of the average result for any variant was larger than ±5—7%, the entire analysis was repeated.

Experimental results

(A) First part of the experiments involving absorption of the active subsctances and live-wilting

In the course of the absorption treatment of the isolated shoots, an interesting change in external disposition occurred in certain variants; this is illustrated in Fig. 1.

From Fig. 1 it may be stated that exogenous IAA and combinations of IAA with other active substances gave rise to almost the same degree of curvature. This curva-

Fig. 1. Absorption treatment of isolated lucerne shoots with water and with solutions of biologically-active substances. The basal parts of groups of shoots weighing 100 g were immersed in the solutions. Absorption took place from 150 ml of solution, for 24 hours. The solutions were changed every 8 hours, and theirt volume changes too were measured. Absorption was carried out in air at 20° C, with a humidity of 60% and under an illumination of 3000 lux. Treatments: 1=water; 2=ABA (5 mg/l); 3=IAA (20 mg/l); 4=IAA+GA_3+FAP (20+50+20 mg/l); 5=IAA+GA_3+FAP+ABA (20+50+20+5 mg/l).

ture of the shoots is not a consequence of the water-deficiency (turgor decrease), for the experimental data revealed that the absorption treatment caused maximum turgor (Table 1). From these IAA-specific epinastic curvatures of the shoots it may be concluded that the IAA solution joined into the metabolism via the absorption circulation, since its effect could be exerted only in this way. It may also be stated that neither the GA_3 administered together with the IAA, nor the FAP and the ABA, antagonized these curvatures at all. In the leaves of the shoots treated with the ABA solution, the state of the stomata was checked by microscopic study at 2-hourly intervals. Even after 6 hours' absorption, the ABA had caused all of the stomata of the leaves to close completely. This fact proves beyond any doubt the absorption, distribution and efficiency of ABA.

The amino acid analyses revealed that the total amino acid content of the shoots fixed and dried immediately after cutting-off is very low (18.3 mg/g dry matter), and compared to this the strong water-deficit lasting for 2 days after the water-absorption, i. e. the live-wilting, gave the greatest change, as the total amino acid content increased to 81.5 mg (4.45 times that of the fresh control). The water-treated and live-wilted variant is a control compared to the variants treated with the active substances, and if the IAA solution absorbed variant is compared to this it may be stated that its total amino acid content increased still further, by about 20% (to 97.9 mg).

At the same time, compared to the water-treated control, the total amino acid content did not change significantly on the action of ABA, IAA+GA_3 +FAP and IAA+GA_3+FAP+ABA treatments: 83.2, 81.8 and 82.7 mg/g, respectively.

It may be concluded from the results that the effect of IAA in increasing the amino acid synthesis and accumulation, on the occasion of the water-deficit of the isolated shoots, is cancelled out by the GA_3, the FAP and the ABA jointly. At the same time, however, the shoots treated with ABA and with ABA+IAA+GA_3+FAP absorbed approximately 30% less liquid during the 24-hour illumination; as demonstrated by the microscopic examinations, this was induced by the ABA bringing about complete closure of the stomata. The closure of the stomata on the action of ABA was not antagonized by the joint effect of IAA, GA_3 and FAP either. The same

finding was made by TUCKER and MANSFIELD (1971), but they did not investigate the amino acid changes.

The first part (A) of our experiments has two main results: (i) at the time of a water-deficit, a solution of exogenous IAA with a concentration of 20 mg/l increases the total amount of free amino acids accumulating considerably (by 20%); (ii) an ABA solution containing 5 mg/l decreases the water-uptake of the shoots by about 30%, by closing the stomata, thereby reducing the transpiration.

(B) Second part of the experiments involving absorption of the active substances and live-wilting

In the second absorption experiment, besides pure solutions of IAA and ABA, the joint effect of the two active substances is also studied, with the idea that the IAA may increase the accumulation of free amino acids to an even higher level in the live-wilting via the transpiration-decreasing effect of the ABA. The water household and dry weight data and the soluble total protein contents are listed in Table 1.

It can be seen from Table 1 that, as a result of the exogenous ABA, the shoots absorbed nearly 30 ml less water in one day than the control immersed in water, or

Table 1. Effects of IAA, ABA and IAA+ABA on the water balance, dry weight and soluble total protein content of isolated lucerne shoots in the event of a strong water-deficit. Absorption of water or of solutions of biologically-active substances lasted for 24 hours, and the water-deficit for the next 2 days (in air at 20 °C, with a relative humidity of 60% and under an illumination of 3000 lux).

Treatment of isolated lucerne shoots	100 g shoots (fresh weight)				Soluble total protein, mg/g live weight
	Amount absorbed in 1 day from water or solution, g	Amount bound in 1 day from water or solution, g	Weight after 2 days' live-wilting, g	Dry weight after 2 days' live-wilting, g	
Fixed immediately on cutting-off (control)	—	—	—	22,6	25,7
Absorbed with water for 1 day, then live-wilted	121,3	12,5	43,2	22,3	24,9
Absorbed with ABA solution (5 mg/l) for 1 day, then live-wilted for 2 days	83,8	15,1	47,5	22,6	25,2
Absorbed with IAA solution (20 mg/l) for 1 day, then live-wilted for 2 days	112,7	15,4	46,7	22,4	24,8
Absorbed with ABA+IAA solution (5+20 mg/l) for 1 day, then live-wilted for 2 days	82,0	15,5	47,9	22,7	25,6

the shoots treated with exogenous IAA. At the same time, as regards the amount of water bound the differences are not large. At any event, the essential fact is that the IAA administered together with the ABA did not cancel out the significant effect of ABA in reducing the water turnover when applied alone.

It may also be stated from Table 1 that the dry weight and soluble total protein content of the isolated lucerne shoots treated with the active substances did not display a characteristic difference compared to the (fresh) control fixed and dried immediately after the cutting-off. It may further be established from the Table that the protein content of the shoots does not change essentially either during the live-wilting if the photosynthesis is active in the incubation period, or the isolated shoots possess a satisfacory carbohydrate basis. The illumination and functioning photosynthesis are therefore very important factors in the course of live-wilting (PÁLFI et al., 1974ab, 1975ab).

A paper chromatogram developed with isatin, which forms the basis of the colorimetric determination of proline, is illustrated in Fig. 2. The isatin reagent reacts extremely sensitively with proline to give a dark blue colour, whereas with other amino acids it gives only pale pink or reddish-brown spots, and accordingly these latter have been encircled.

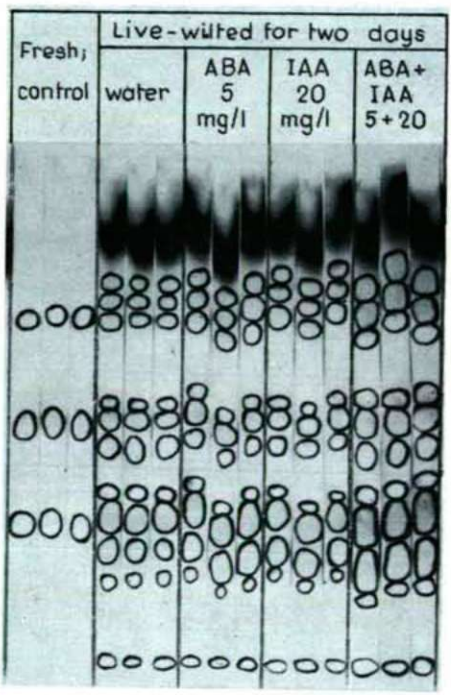

Fig. 2. Changes in the free amino acid composition of isolated lucerne shoots as a result of absorption of water or active substances and strong water-loss (live-wilting) for 2 days. An ascending one-dimensional paper-chromatogram, run in phenol-water solvent and developed with isatin reagent, for elution and colorimetric measurement of proline (every variant run in triplicate, or in 3 bands).

It emerges from Fig. 2 that free proline can be detected only in traces in well-watered shoots which were fixed and dried immediately after isolation (fresh control). At the same time, the proline has accumulated to an extremely large extent in shoots exposed to a strong water-loss (live-wilted) for 2 days. The colorimetric data showed that, of the four variants, the ABA+IAA treatment gave the highest proline content; the absorption treatment with IAA gave a value close to this, but somewhat lower.

Figure 2 indicates that the total amino acid content of (fresh control) shoots fixed immediately after cutting-off is very slight, and that, as regards the water-loss variants, the shoots treated with ABA+IAA gave the largest and darkest spots, i. e. the total amino acid concentration of this variant was the highest.

In the course of the experiments, a detailed analysis was also carried out on the amino acids. The results are presented in Table 2.

It can be seen in Table 2 that as a consequence of the strong water-loss for 2 days (live-wilting under illumination) the total amino acid content of the shoots saturated

Table 2. Changes in the free amino acid content of isolated lucerne shoots as a result of exogenous ABA, IAA and IAA+ABA solutions, and a strong water-loss (live-wilting) for 2 days. Detailed amino acid composition.

Abbreviation for amino acid (+ = indispensable)	Dried on cutting-off (fresh control)	Dried after 2 days' live-wilting			
		Water	Active substances, mg/l		
			A B A 5	I E S 20	ABA+IES 5+20
	mg/g dry matter				
Asp	0,61	1,46	1,52	1,55	2,15
Thr+	0,63	2,81	2,26	2,31	3,02
Ser	1,72	4,77	5,63	4,83	4,81
Asn	5,31	18,28	18,80	20,32	37,87
Gln	1,65	4,52	4,50	5,61	6,70
Pro	0,40	15,03	14,11	18,14	20,68
Glu	0,82	1,54	1,58	1,46	1,83
−Amb	0,10	0,35	0,47	0,51	0,62
Gly	0,26	0,41	0,40	0,47	0,56
Ala	2,56	4,02	4,71	6,10	5,04
Val+	0,77	3,37	3,52	5,42	5,38
Cys	0,09	0,13	0,25	0,20	0,16
Met+	0,12	0,63	0,68	1,17	1,03
Ile+	0,64	2,64	2,73	3,14	5,87
Leu+	1,02	3,50	3,69	4,21	6,89
Tyr	0,51	1,13	1,02	0,98	2,10
Phe+	0,65	2,52	2,70	3,19	4,75
Trp+	0,42	0,90	0,91	1,86	1,64
Lys+	0,54	1,64	1,67	1,82	1,73
His+	0,20	1,81	1,74	2,43	2,22
Arg+	0,68	1,44	1,50	1,65	2,26
Total aminoacid, mg/g	19,70	72,90	74,39	87,37	117,31
As a percentage of the dry matter	1,97	7,29	7,44	8,74	.11,73

The error is less than ±5%.

with water after the isolation increased to 3.70 times that of the freshly-fixed control. From this fact it may again be stated that the most significant change in our experiment was in the total amount of amino acids forming and accumulating at the time of a strong water-deficit (including the amounts of proline and asparagine).

In the treatment of absorption with water, the greatest increase was found for proline, which rose in amount to 37 times that for the freshly-fixed shoots. Naturally, the lucerne exhibited a proline type now too, since the strong water-loss caused the proline in the shoots to increase above 1.0% in every live-wilted variant. As found by both ourselves and others, the accumulation of a larger quantity of proline promotes the tolerance of the water-deficit in mesophytic, soft-stemmed plants (KUDREV and TYANKOVA, 1966; PÁLFI, 1968ab, 1969; LEWITT, 1972; VALLEE, 1973; HUBER, 1974; PERDRIZET, 1974; WALDREN and TEARE, 1974; BATES et al., 1975; MELIN, 1975; etc.).

In the shoots absorbed with water and live-wilted there is also a very considerable accumulation of asparagine; although this increased to be only 3.4 times the asparagine content of the (control) variant fixed immediately after the cutting-off, in absolute terms it exceeded even the proline. It appears that, in the even of a water-deficit, lucerne is not only of proline-accumulating type, but also of asparagine-accumulating type. This conclusion was reached by IZMAYLOV et al. (1974) too.

Besides the proline and asparagine, however, the concentration of the essential amino acids also increases considerably as a result of live-wilting, in comparison with the (fresh control) variant fixed immediately after cutting-off: e. g. in the shoots absorbed with water the 2-day strong water-deficit led to an increase of 4 times in the threonine, valine, isoleucine and phenylalanine, an increase of 5 times in the methionine, an increase of 3 times in the leucine and lysine, an increase of 2 times in the tryptophan and arginine, and an increase of 9 times in the histidine.

Since we primarily wished to determine whether the biologically-active substances applied lead to changes in the composition and total amount of the amino acids formed as a result of the live-wilting of the shoots under illumination, in the following the results are referred not to the variant fixed immediately after cutting-off, but to the variant saturated with water for 1 day and subsequently live-wilted for 2 days.

From the total amino acid quantities in Table 2 it can immediately be established that the exogenous active substances changed the amino acid picture of the (control) variant treated only with water and then subjected to a strong water-loss for 2 days. The IAA treatment and the IAA+ABA combination increased the total amount of amino acids by 19% and 60%, respectively, compared to the water-treated control. At the same time, the ABA treatment in itself did not result in a characteristic amino acid change. Our assumption was therefore confirmed: the ABA retained its water turnover (transpiration)-decreasing effect even when combined with IAA, and in addition (or even thereby) elevated the amino acid-increasing effect of IAA by a factor of 3, from 19% to 60%.

Table 2 also shows that the combined ABA+IAA treatment led to a significant increase in the concentration of the amides, proline and the essential amino acids, compared to the water-treated shoots; as regards the protein synthesis, therefore, the amino acid composition improved further. It may be seen from the data that ABA applied alone did not produce an appreciable change, either qualitatively or quantitatively, in the amino acid metabolism level; it therefore acted by another route or

directly on the water turnover (e. g. by closing the stomata). This conclusion was also reached by Mittelheuser and Van Steveninck (1969), Tucker and Mansfield (1971), Kriedemann et al. (1972) and Lewitt (1972).

Discussion

In our previous work (Pálfi, 1971; Pálfi et al., 1972, 1973, 1974, 1975) it was found that the increase of the total free amino acid content of the shoots and leaves isolated from greenstuffs and green fodder is generally of different extents in the course of live-wilting, depending on whether or not the species in question is of proline-accumulating type. With the method of artificial live-wilting, the protein-forming total amino acid content of species of proline type (e. g. lucerne, clover, pea, wheat, savoy cabbage) can be increased to 7—11% of the dry matter. At the same time, the total amino acid concentration of species of non-proline type (e. g. spinach, lettuce, maize, bean, white lupine) generally attains a somewhat lower level, 5—8%, in the course of live-wilting under illumination.

The higher total amino acid accumulation of the species of proline type originates primarily from the large amount of proline itself, this generally being accompanied by a considerable asparagine concentration increase too, particularly in the Leguminosae family species of proline type. Live-wilting does not lead to a significant difference in general between the species belonging to the two types, as regards the appreciable increase in the total amount of essential amino acids (Pálfi et al., 1974, 1975).

The total amino acid level and the qualitative composition attainable during live-wilting depend on many other endogenous and exogenous factors in addition to whether the given plant species is of proline type or not.

A factor which may be regarded as endogenous is the selection of the appropriate varieties within the species in question, and the selection of the various developmental phases of the plant. It seems that optimum choice and adjustment of the exogenous factors are both of very great importance. In addition, it must also be taken into consideration that a change in the value of some exogenous factor may lead to subsequent changes in the levels of the other exogenous effects.

As regards the exogenous conditions, stress must primarily be laid on the importance of the fact that the plants destined for live-wilting should be field-grown under optimum conditions. This ensures a favourable physiological state of the isolated shoots. During the pre-growing, the provision of the plants with a good supply of water, nitrogen and other nutrients is in particular indispensable. It is also essential that the weather for 4—5 days prior to the cutting-off of the shoots should also be relatively favourable: it should be sufficiently warm, and the plants should receive several hours of sunlight daily. In this case, the shoots possess a satisfactory carbohydrate reserve during the live-wilting, and thus "protein respiration" does not occur. If the nitrogen supply of the plants is not certainly favourable, a plant nutrient spray may be applied too 2—3 days before the shoots are cut off.

The season in which the individual plant varieties are grown is also of importance. For example, as a result of live-wilting, spring spinach always accumulates more total amino acid than any variety sown in autumn. Greenhouse-grown spinach, however,

gives a very poor result. Lucerne and savoy cabbage harvested in the summer provide a better starting basis than those harvested in the autumn.

During live-wilting it is most important to regulate the following exogenous factors: the air temperature; its relative humidity; its carbon dioxide content; the nature and intensity of the illumination; the duration of live-wilting; and the rate and extent of loss of water of the isolated shoots. On attainment of the optimum duration of live-wilting, the shoots must immediately be fixed at 70 °C and dried, as protein loss will occur in subsequent live-wilting.

The fact that fairly different levels of amino acid accumulation were obtained in the live-wilting of a given plant species and variety is atributed to the varying conditions of pre-growing, and to the variations in the exogenous factors of live-wilting. This is not only characteristic for the results of our earlier work (PÁLFI et al., 1972, 1974, 1975), but also emerges from the two parts (sections IIIA and IIIB) of our present experiment.

As regards the amounts of total amino acid accumulating during live-wilting, however, the differences due to the environmental conditions are not completely extreme: in the case of greenstuffs such as spinach and savoy cabbage, we ourselves did not grow the starting raw material in even a single case, but obtained it commercially, and accordingly, as a rule, neither the variety of the plant, nor its site of growth, and indeed in many cases the exact time of harvesting, was not known. Nevertheless, in the live-wilting of these isolated leaves fairly uniform total amino acid accumulation results were achieved (PÁLFI, 1971; PÁLFI et al., 1972).

In further of our experiments (PÁLFI et al., 1975), physiological factors were sought, the application of which led to a further enhancement of the total amino acid accumulation in the course of live-wilting. We set out from the fact that the endogenous ABA content of the isolated leaves is elevated to 10—40 times the normal level as a result of a water-deficiency persisting for some hours (WRIGHT, 1969; ZEEVAART, 1971; KRIEDEMANN et al., 1972). At the same time, as a consequence of an increasing water-deficit, the concentrations of IAA and other growth substances decrease rapidly (DARBYSHIRE, 1971; ITAI and VAADIA, 1971).

The data of ASPINALL et al. (1973), BATES et al. (1973), HUBER (1974) and KANG and STANLEY (1971) indicate that there is a correlation between the activity changes of the individual growth substances, the accumulation of the amino acids and the water-household of the plants, and the endurance of the water-deficit.

It can be seen from all these data that the biologically-active substances play a regulatory role as regards the amino acid and protein metabolisms in the course of the water-loss and live-wilting of the isolated shoots. Positive results as regards the magnitude of the accumulation of amino acids were achieved earlier in live-wilting experiments, by the application of certain growth substances exogenously (PÁLFI et al., 1975).

The present experimental results show that exogenous IAA or combined IAA+ +ABA treatment during the live-wilting of isolated lucerne shoots leads to an enhancement of the accumulation of protein-forming amino acids by a further 20—60%, without an accompanying substantial decrease in the protein content, if the shoots possess a satisfactory carbohydrate reserve (under illumination). At the same time, compared to the levels for the water-treated control, the total amino acid values of the isolated shoots were not increased during live-wilting by treatment with ABA alone, or by the combination ABA+IAA+GA_3+FAP. The ABA treatment decre-

ased the water-turnover of the shoots by 30%, by closing the stomata, and this effect was not antagonized by the joint effect of IAA, GA_3 and FAP.

In the evaluation, attention must be paid to the fact that the total amino acid content of the shoots fixed and dried immediately after cutting-off (fresh control) is very low; for instance, in part (A) of our experiment it was 18.3 mg/g dry matter. The largest change compared to this was given by the strong water-deficit for 2 days, i. e. live-wilting, since the total amino acid content then rose to 81.5 mg/g dry matter, 4.45 times the value for the fresh control. This is at present the highest free amino acid production attainable by biological means.

The amino acids necessary for the building-up of the proteins in the organisms of animals and man are mainly taken up in the form "bound" into proteins. However, hydrolytic splitting of the proteins, i. e. digestion, is accompained by a significant protein loss.

According to the data processed by SZLAMENICZKY (1972), for all the animal species in Hungary the total amount of digestible protein in the fodders used yields on average only 18—19% animal protein. This means that not even one-fifth of the protein content of the fodders undergoes protein-utilization. In contrast, the free protein-building amino acids of plants are absorbed to an extent of 98%, i. e. almost totally (RIGÓ, 1976).

In exact human nutritional examinations (RIGÓ, 1976), it emerged that the digestibility quotient for an amino acid mixture is 98, while that for proteins is only 37; in enteritic children the protein-utilization as regards the free amino acids is 90, whereas that for proteins is only 26. Feeding with free amino acids is therefore of great importance in connection with dietetics too. BARNA et al. (1976ab) report that the utilization of amino acids in healthy individuals is more advantageous that that of proteins; the digestibility quotient, the biological value and the protein-utilization uniformly exhibit higher values. RIGÓ assumes that our foods will be subjected to new judgement from the aspect of the variations in the free amino acid content.

As found by THOMAS (1973), the value of lucerne is increased by the fact that it may also be used for human feeding. In many states in the USA, lucerne is already sold in the form of large tablets in numerous stores, and recipes have been developed for the preparation of bread and other foods. Thomas reports that the experience to date shows that lucerne is a very effective nutrient, with which many pathological physiological lesions too may be treated. In addition, lucerne is an important constituent of baby foods.

We too consider that young lucerne shoots, carefulls dried at 70°C and granulated, and possessing an elevated amino acid content as a result of this live-wilting, could form a sure protein basis in the feeding of populations in protein-poor countries. In Hungary a small group has been regularly consuming green-vegetable foods prepared from live-wilted lucerne or a powdered lucerne and spinach mixture (flavoured in varion ways) for 3 years.

At present, as RIGÓ (1976) too concludes, encouraging methods for the future are the production of free amino acids with yeasts, the production of amino acids from side-products of the petrochemical industry with pure cultures of bacteria, and the accumulation of the amino acids in isolated leaves.

We have established that an extract can be obtained from lucerne or savoy cabbage by simple boiling with water, the amino acid content of which is enriched to 8—10% of the dry matter by live-wilting, and that a concentrate containing 40—50%

amino acid can be prepared from this by evaporation. At present the live-wilting of isolated shoots and leaves is the most simply performable procedure, and therefore the cheapest method for the production of natural amino acid mixtures by biological means.

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