

MULTIPLICATION OF THE ESSENTIAL AMINO ACIDS DURING THE LIVE-WILTING OF LEAVES

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The physiological processes in connection with the loss of water resp. wilting of leaves and shoots are little known although wilting is a common phenomenon in nature and practical life. Wilting takes place from the beginning of the cutting off of the green vegetables in the fields until the beginning of their ingathering, storing, commercial distribution and processing (spinach, garden sorrel, cabbage, common lettuce, etc). Savoys and garden cabbages gathered in great quantities in autumn lose their water gradually, i. e., they wilt during the winter storage. From the harvesting of fodder crop until their wilting in the open field, as well as during the processing of silage crops, too, the shoots separated from their root system continue wilting for a while.

Several researchers dealt with investigating the physiological processes during the water deficit, i. e. wilting of plants (KEMBLE and MACPHERSON, 1954; RADENKOVA, 1963; PETINOV, 1963; CHEN et al., 1964; BRITIKOV, 1965; BARNETT and NAYLOR, 1966; STEWART et al., 1966; GENKEL et al., 1967; DOVE, 1967; MARANVILLE, 1967, etc.). The practical usefulness of their findings, however, is often difficult to be realized since they carried out their experiments on various species and sorts of plants, on the leaves either of field plants or on those in culture pots or of intact ones, or on isolated leaves. Furthermore, even the same plant species were processed at different degrees of developments or grown under different external conditions.

In our experiments, we were interested, from among the physiological processes occurring in the course of the water deficiency of plants, first of all in the amino acid metabolism (PÁLFI, 1968, 1969; PÁLFI and JUHÁSZ, 1969, etc.). It is known that the free amino acid content of plants, resp. leaves is considerable, as compared to that of animal tissues, representing 1.5—1.8 percent of the dry matter. In case of plants, however, the ratio of the essential amino acids to the basal amino acids — both at free amino acids and in those bound into proteins — is unfavourable in regard to human alimentation.

The present investigations aimed at ascertaining whether the essential amino acid composition of the fresh leaves with an optimal water-balance or that of wilting isolated leaves is more similar to the human tissue extracts.

A further aim was to clear up how the dry matter, proline and total amino acid content as well as the amount of the soluble total protein, of isolated leaves develop in the light and the dark, while they lose water.

It was also examined whether during the water loss of the leaves isolated and infiltrated with 2,4-dinitrophenol the quantity of proline is growing, incubated in light, and how the abnormally high proline content of leaves is influencing that process.

In agriculture and in the practice of food industry it is important to know, under what conditions these plants have the most favourable nutriment composition during the storage of vegetables, the preservation of fodder-crops, hay-harvesting and ensilation.

Materials and Methods

The fully developed leaves of garden sorrel (*Rumex scutatus* L., variety, „Kerti”) and of spinach (*Spinacia oleracea* L., variety, „Viroflay”) were gathered at the end of the vegetative developmental phase. The garden cabbage for meals that was already capitata (*Brassica oleracea* L. var. *capitata*) was dissolved to its leaves for promoting its wilting. The shoots of lucerne (*Medicago sativa* L., variety, „Lilavirágú”) and the leaves of sunflower (*Helianthus annuus* L., variety, „Kisvárdai”) were collected before the beginning of gemmation, and the shoots of maize at the age of 6—8 leaves (*Zea mays* L., variety, MVDC 300).

The water supply of the experimental field plants was for a rather long while at an optimum level in the time before sampling. The fresh and wilted variants of leaf-samples were previously apportioned into equal doses of 100.0 g fresh matter, in three repetitions. We have then dehydrated the variants putting them, after rinsing in tap water, on filter-papers, and incubating the leaf-groups of determined weight in the light or the dark for two days (at 25 °C, and a relative humidity of 70 percent). The control variants were fixed at 60 °C immediately after being rinsed, and weighed after being dried to constant weight.

For two days we have registered wilting weights in case of live-wilted variants, for being able to refer the results of protein detections to identical bases. The wilted variants were also dried at 60 °C, and the whole material was pulverized.

The qualitative and quantitative demonstration of amino acids was carried out by starting from the same amount of dry matter. One- and two-dimensional layer- and paper-chromatograms have been prepared from the extracts of every sample. The solvent of the first dimension was butanol-acetic acid-water (2 : 1 : 1), that of the second one phenol-water (4 : 1). The reaction to ninhydrin was fixed by copper-salt solution. The quantitative measurements of eluates, too, were carried out from copper complexes. The total amino acid determinations were performed with the comparative standards of eluates already published (PÁLFI, 1964a, b). The total amino acid data were checked up with Rosen's colorimetric procedure (1957), too. The proline measurements were accomplished with the isatin-colorimetric method and according to Chinard (1952). The soluble total protein extracted with tris-buffer (pH 7.5) was determined according to Lowry et al. (1951). During this procedure we have reduced the fresh and wilting weights to dry-weight, to get a real basis for comparison.

Experimental results

First we have studied in a many-sided way the effect of water deficiency, exerted on the amino acid metabolism of leaves, only on a single variety of a plant species (paprika, *Capsicum annuum* L. var. *longum* variety, „57—13”). The experiments included field-work and tests in culture media. Then we have investigated, how the members of various plant families, resp. the single plant species and cultivars behave in case of water deficiency and isolation of leaves, if the water loss was not retrieved. For that purpose we have investigated the free amino acid composition of the leaves of (mostly cultivated) plants belonging to 66 plant species, including leaves of *Gymno-* and *Angiospermae*, herbaceous and woody, *Mono-* and *Dicotyledons* including leguminous plants as well (PÁLFI, 1970).

It was found that up to a certain degree of live-wilting a large amount of total amino acid, resp. proline is accumulated in all the leaves investigated. The accumulation of free total amino acid in leaves in such a high degree (200–500 percent) that, owing to the water loss, it is connected with an extraordinary (1000–10 000 percent) increase of proline, was denominated by us an „amino acid metabolism of proline type” (PÁLFI, 1970). The development of the amino acid metabolism of proline type is, therefore, a general regularity, manifested widely in the world of plants, that was demonstrated by us at the most important families of the cultivated plants (*Solanaceae*, *Cruciferae*, *Leguminosae*, *Compositae*, *Gramineae*, etc.), too.

In the following we have endeavoured to find out whether the „amino acid metabolism of proline type” manifests itself exclusively as a result of water deficit or whether it can be caused also by another environmental factor. We have tried culture pot experiments with plants suffering from loss of N, P, K and other nutrients or supplied one-sidedly with plenty of a single nutrient. In addition, we have investigated the free amino acid composition of plants suffering from viruses, fungal or bacterial infections. From the results we have come to the conclusion that „amino acid metabolism of proline type” develops only as a result of water insufficiency. To be sure, in some cases of our comprehensive investigations we have noticed some signs of „amino acid metabolism of proline type” in spite of optimum water content of the soil.

In the course of the careful elaboration of data, however, it has turned out that the water uptake, resp. circulation was inhibited anyway (physiological dryness). Such inhibitor of water supply may be the high total salt content of the soil water in case of alkali soils, and cold soil (and cold air) in case of early plants or those surviving the winter (PÁLFI, 1969; PÁLFY and JÚLIA JUHÁSZ, 1969, 1970).

At any rate, if the total amino acid content of leaves is significantly above the normal level and the proline has increase by several hundred or thousand p. c., it can be ascertained undeniably on the basis of „amino acid metabolism of proline type” that the plant suffers from water deficiency (PÁLFY, 1969).

We hadn't any knowledge, either, in respect of whether „amino acid metabolism of proline type” manifests itself in every organ of plants as a results of water deficiency. For deciding the problem, we have grown wheat, paprika, and sunflower plants (cultivar „Bezostaya”, Spice „57–13”, and „Kisvárdai”). During a provoked strong water deficit the free amino acid extracts of leaves, stem ears, and roots were separately analysed. It turned out that „amino acid metabolism of proline type” manifests itself in any green organ of the plant, i. e., in any one containing chloroplastis, but not in the roots.

This results has suggested that photosynthesis may have a role in the development of „amino acid metabolism of proline type”. For clearing the problem, we have ceased irrigating the culture pot paprika and sunflower plants (variety „57–13” and „Kisvárdai”), and kept them in the dark for ten days. As shown by the analysis of the daily taken leaf samples the proline and total amino-acid content of leaves may temporarily grow for a few days as a result of water deficiency, but in the days before withering the proline content entirely decreases and the free amino acid content drops also to a low level. Photosynthesis or its products have, therefore, a role in realizing, resp. sustaining „amino acid

metabolism of proline type". The development of „amino acid metabolism of proline type" is demonstrated in Fig. 1.

After ceasing to irrigation the culture-pot wheat plants (variety, „Bezostaya"), leaf samples were taken daily for eight days. The extracts of the collected samples have been developed on eight separate strips of a chromatogram-paper. After developing the amino acid spots, we framed the pale spots with a pencil and photographed them (Fig. 1).

On Fig. 1. it can be seen that the darkest and largest spots are made by proline but only from the second day after the watering was stopped (strips CDEFGH). In the leaf extract of the irrigated wheat (strip „A") proline appears only in traces ($0,5 \mu\text{g}$). As a result of water deficiency for seven days, that quantity has augmented 130 times ($65 \mu\text{g}$). On Fig. 1 it can also be seen that, apart from proline, the amount of other amino acids has also increased considerably, (200–500 percent).

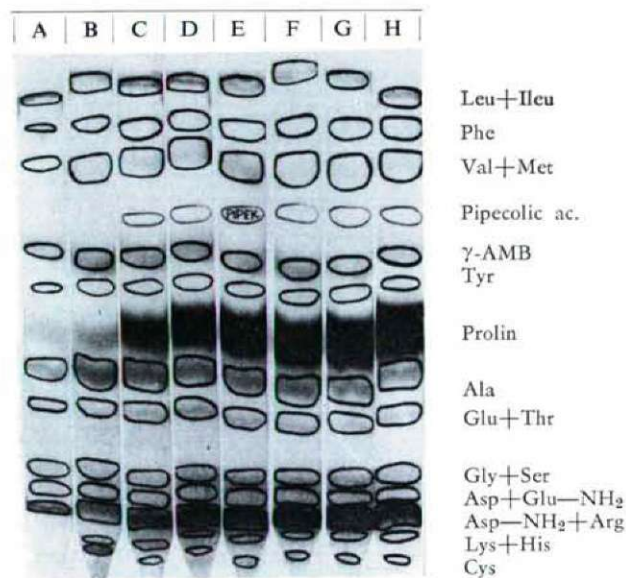


Fig. 1. The effect of the increasing water deficiency on the amino acids of wheat leaves. Culture-pot experiment. (Butanol solution; developer: isatine). „A"=constantly well-irrigated (control). „BCDEFGH"=increasing water deficiency as a result of interruption of the irrigation for 1, 2, 3, 4, 5, 6, 7 days

In the following we have investigated the amino acid spectrum of the leaves detached from the plants and losing water in isolated state. We have ascertained that in the leaves losing water because being separated from the shoot, „amino acid metabolism of proline type" also develops like in the leaves of normal plants if they wilt exposed to light. Hence it follows that the wilting of isolated leaves is also a physiological process during which some biochemical changes similar to those in the leaves of intact plants become realized as a result of a strong water deficiency. An essential difference is that the amino acid accumulation, that developed during 3–6 weeks of water deficiency in

the normal field plants, takes but 3—4 days in leaves losing water in isolated condition. For emphasizing the water loss, in live state resp. wilting of the isolated leaves, we have denominated this phenomenon „live-wilting” (bio-dehydration). If the isolated, live-wilting leaves get light — they assimilate, meanwhile respiring — they dissimilate, generate energy, and consume. During live-wilting the water, dry matter, protein and carbohydrate content of the isolated shoots and leaves changes. The live-wilting depends therefore, (in a positive or negative way) on the production of organic matter, too. During live-wilting the proteinbuilding amino acids accumulate considerably and the proline concentration reaches an extremely high level.

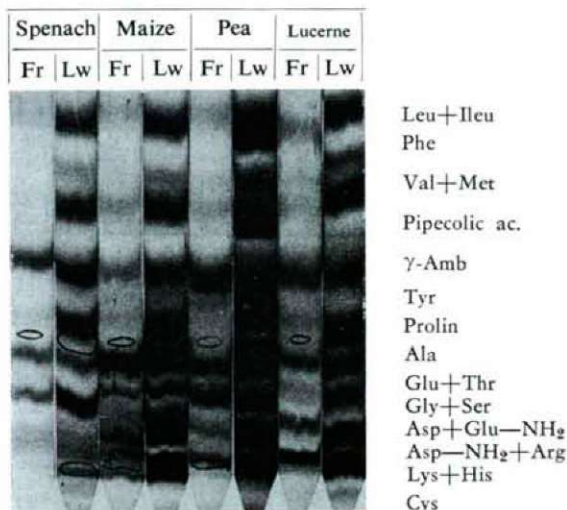


Fig. 2. Amino acids of leaves fixed and dried at isolation (fresh), as well as those dried after live-wilting for three days. Developer: ninhydrine. Fr=Dried fresh (control) Lw=Dried after live-wilting

It may supposed that with an artificial live-wilting process, as a result of a high-degree accumulation of free amino acids, green vegetables and animal fodderplants of concentrated protein value can be obtained. On the neighbouring strips of the chromatogram in Fig. 2, the extracts of the fresh and of the artificially live-wilted isolated leaves of the same plant occur alternately.

As shown by Fig. 2, the free amino acid content of the leaves fixed after the artificial live-wilting (LW), many times exceeds that of fresh leaves fixed immediately at isolation. That is particularly obvious in the upper part of some strips of the chromatogram where the spots of leucine + isoleucine, phenylalanine, valine and methionine is located. From the extracts of fresh leaves (Fr) these amino acids can be demonstrated in traces only. The essential amino acids occur in the fresh leaves in but a small amount. The ratio of essential and basic amino acids considerably changes owing to the live-wilting of the isolated leaves, as shown on Fig. 3.

It can be established from size and colour intensity of the spots of Fig. 3. that the free amino acid composition of the live-wilted pea and spinach leaves

resembles much more to the amino acid spectrum of human tissue extracts than that of fresh leaves. The leaves wilted by an artificial water deficiency approach more the ratio of the amino acids of human tissue extracts by increasing considerably the amount of a few essential amino acids (leucine + isoleucine, phenylalanine, valine + methionine, threonine, arginine, histidine and lysine). From the spots of Fig. 3 we can draw also the conclusion, that the total amino acid content of the live-wilted leaves is a manifold plural of that of fresh leaves fixed at isolation.

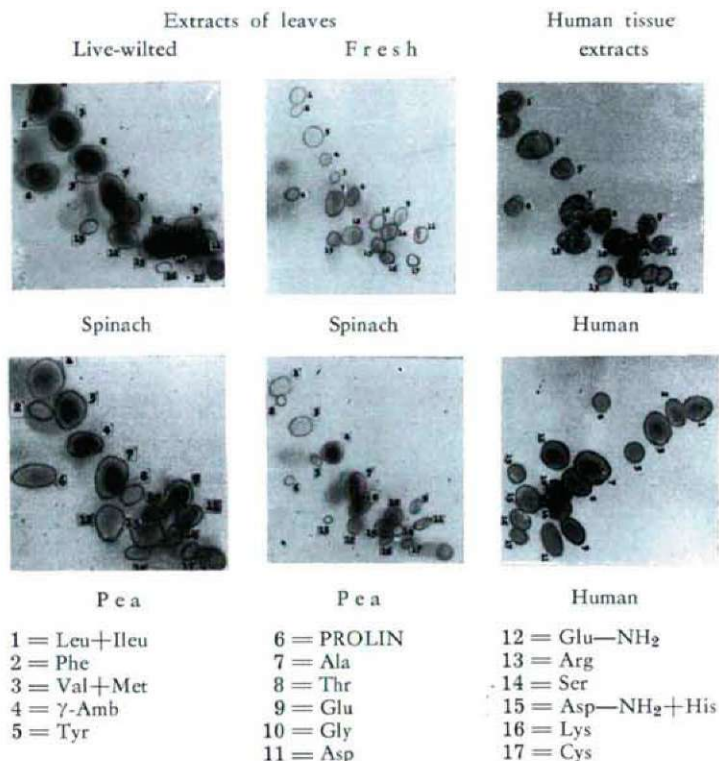


Fig. 3. Free amino acids of plant and human tissue extracts

The quantitative data of the amino acid increase appearing during live-wilting are published in Table I.

Isolated shoots of maize and lucerne and leaves of the other plants were wilted.

It can be seen on Table I that the proline content of the cabbage and sunflower leaves, live-wilted in light, exceeds that of fresh leaves more than a hundred times. At the same time, the proline accumulation of the leaves of garden sorrel and spinach does not reach the tenfold value of the fresh variant. In the degree of proline increase there are therefore, considerable differences

according to plant species. It is worth mentioning, too, that garden cabbage, paprika, wheat, and sunflower, we could obtain even 6–7 percent pure free proline content, as a result of a live-wilting in light for 4 to 5 days (referred to dry matter). This quantity can easily be obtained after boiling in distilled water. It may be ascertained, anyway, that the proline accumulation is considerable in each of the plants investigated, as compared to the fresh control it is at least 500 per cent.

Table 1

Change of the free proline and total amino-acid contents of isolated leaves, as a result of live-wilting in the light and in the dark.

Garden cabbage and maize wilted alive for three days, the other plants for two days.

Plants	Proline				Total amino acid			
	mg/g dry matter			Per cent increase in dry matter of fresh leaves; light	mg/g dry matter			Per cent increase in dry matter of fresh leaves; light
	Fresh leaves	Live-wilting leaves			Fresh leaves	Live-wilting leaves		
	light	dark			light	dark		
Garden sorrel	0,32	1,7	0,9	531	15,7	47,6	43,2	303
Spinach	0,38	2,2	1,3	578	16,2	68,6	56,5	423
Garden cabbage	0,40	42,4	32,7	10 600	16,3	92,8	68,4	569
Maize	0,29	12,8	9,3	4413	20,2	66,3	54,1	328
Lucerne	0,46	15,3	8,4	3326	17,1	57,2	50,3	334
Sunflower	0,25	26,6	10,8	10 640	12,6	41,5	32,7	329

The total amino acid is to be understood without asparagine but with proline.

Deviation of standard error of the three repetitions of the variants is less than ± 8 per cent.

Table I shows also, that in the plants investigated the increase of the total amino acid, owing to the live-wilting in the light, is of very high degree, between 300 and 600 percent (in the percentage of the fresh matter).

Such outstanding increase of the amino acid contents can be achieved even by live-wilting only in case of leaves that are well supplied in regard to mineral nutrition and have due carbohydrate reserves, but not in case of under-nourished and weakened plants. At our live-wilting experiments carried out frequently with garden cabbage, we have obtained more than once 10 p. c. total amino acid contents. A garden cabbage like that can be considered in itself as an amino acid concentrate since 1 kg dry matter contains 100 g free amino acid.

It can be seen on Table I that the proline and total amino acid content of leaves live-wilted without light did also considerably increase as compared to that of the fresh ones. The accumulation of amino acid in the dark is, however, considerably smaller in case of all the six types of the plants investigated that that of the variants wilted in the light. Other experimental results (PÁLFI, 1968, 1969) show that in the next days of the lightless live-wilting even this quantity decreases and, until the time of full drying, it drops to the level of control.

In the following we are investigating the changes in dry matter and soluble total protein accompanying the increase of the total amino acid during the live-wilting (Table II). At weighing the dry matter we have weighed previously, in three repetitions, 100,0 g doses of fresh matter for every variant; then, after two days exposition in the light or in the dark, the doses were fixed and dried.

Table 2

Change of the dry matter and the soluble total protein contents of isolated leaves, as a result of live-wilting in the light and in the dark.

Garden cabbage and maize wilted alive for three days, the other plants for two days.

Plants	Dry matter from 100 g fresh matter				Soluble total protein mg/g fresh matter			
	Fresh leaves; g	Live-wilting leaves, g		Change in the p. c. of the fresh leaves; light	Fresh leaves; g	Live-wilting leaves g		Change in the p. c. of the fresh leaves; light
		light	dark			light	dark	
Garden sorrel	14,06	14,76	13,82	+4,9	16,4	16,3	15,7	-0,6
Spinach	10,25	9,88	9,58	-3,6	20,2	20,5	19,8	+0,4
Garden cabbage	9,84	9,62	9,54	-2,2	9,4	9,1	8,6	-3,2
Maize	16,25	16,96	16,03	+4,3	17,9	18,5	16,5	+3,3
Lucerne	22,68	22,35	21,54	-1,5	32,6	31,9	29,7	-2,2
Sunflower	12,36	13,08	12,10	+5,8	12,3	12,2	11,7	-0,9

The records of total protein of the live-wilted variants take into consideration the water loss as compared to the fresh ones.

It appears from Table II that during live-wilting in the light the dry matter of leaves increases in three plants but decreases in other three species. The change is, however, so small as compared to the control that, with consideration to the maximal deviation of standard error (± 5 p. c.), it is not significant (except the sunflower). At the variants incubated in the dark, however, the dry matter of all the six species decreased more or less. We have obtained similar results from the investigations of the soluble total protein, as well. During a short exposition (two days) in the light the water-loss, inducing high-degree increase of the total amino acid, did not cause any significant decrease in protein.

The soluble total protein content of the variants incubated in the dark, parallel with the dry matter, has decreased in case of all plants investigated. From that we can draw the conclusion that a considerable decrease in dry matter implies a decrease in protein. It may be established that the light, resp. the photosynthesis has, also in case of leaves living isolated and losing water, a considerable part in regard to the quantitative changes in total amino acid, dry matter and soluble total protein.

In the course of our experiments, we have infiltrated isolated tobacco leaves (variety, „Szabolcsi”) with the 100 ppm aqueous solution of 2,4-dinitro-

phenol. According to the results of analyses carried out daily during the live-wilting in the light, there was no considerable increase of proline in any day till the full withering (PÁLFI, 1968a, 1969a, c). At the same time, in the control leaves infiltrated by water, the „amino acid metabolism of proline type” developed already in the second-third day of live-wilting, and we have demonstrated a significant increase of proline even at the full withering of leaves. On the basis of our experimental results we suppose that in the course of live-wilting the precursor of the large quantity of proline is glutamic acid (PÁLFI, 1968a, b) formed from carbohydrates by means of oxidative phosphorylation. This pathway is, however, uncoupled by 2,4-DNP.

We have also performed experiments in the course of which we live-wilted isolated tobacco leaves for two days. Then it was shown that a considerable amount of free proline has already accumulated. The already live-wilted leaves, with high free proline content (1,0 p. c.), infiltrated by the solution of 2,4-DNP, were again live wilted in the light. The quantity of free proline went on growing (2,0 p. c.).

The experiment was carried in by infiltrating further isolated tobacco leaves with the aqueous solution of proline (0,5 p. c.). Beginning next day, we live-wilted the same leaves in the light for three days, after infiltrating them with the solution of 2,4-DNP, increasing in that way the proline content of leaves from 0,5 to 2,3 per cent (as referred to dry matter). In case of a considerable proline reserve of the isolated leaves, therefore, or by means of proline infiltration, 2,4-DNP has no uncoupling effect on oxidative phosphorylation during live-wilting.

As established, „amino acid metabolism of proline type” manifests itself in any phase of development of the plants (PÁLFI, 1969a, b, c); but the largest amount of proline and total amino acid accumulates in the „critical period of water supply”. In case of herbaceous plants this period falls on the time of microsporogenesis (FRENYÓ, 1959; PETINOV and BERKÓ, 1965; SZALAI, 1968; SATILOV and IKONNIKOV, 1969).

Discussion

We have studied the free amino acid composition of the leaves of *Gymno-* and *Angiospermae*, herbaceous and woody, *Mono-* and *Dicotyledons*, (mostly crop plants) belonging to 66 species, in case of water deficiency (PÁLFI, 1968a, b; 1969a, b, c; PÁLFI, 1970; PÁLFI and JUHÁSZ 1970).

The accumulation of the free total amino acid in leaves in such a high degree (200–500 percent) that involves an extraordinary (1000–10 000 percent) increase of proline, was denominated an „amino acid metabolism of proline type”. The „amino acid metabolism of proline type” is a normal phenomenon, current in the plant kingdom. In the course of our multi-sided investigations we have ascertained that „amino acid metabolism of proline type” develops only as a result of water deficiency or of physiological dryness, in case of cold soil or of a high salt content of the soil water (MARIA GOAS, 1968; HEBER, 1969; PÁLFI and JUHÁSZ, 1968, 1969, 1970).

It was proved that the demonstration of „amino acid metabolism of proline type” of plants may be used also for diagnostic characterization of the water

deficiency of plants (PÁLFI, 1969b) and for selecting drought resistant plant types.

We have also ascertained that „amino acid metabolism of proline type” manifests itself only in the green organs of plants containing chloroplasts; but not in the roots. The results of the culture pot water deficiency experiments carried out in the light and in the dark have demonstrated that photosynthesis or its products have some role in realizing, resp. preserving „amino acid metabolism of proline type” (PÁLFI, 1968b, 1969a, c; MORRIS et al., 1969).

Since „amino acid metabolism of proline type” occurs also when isolated leaves are losing water in the light, the wilting of leaves isolated from plants is also a physiological process. By „live-wilting” we mean the continuous, „increasing water deficiency” of isolated leaves or shoots for two-three days (biodehydration). It was found that with an artificial live-wilting process, as a result of a high-degree accumulation of free amino acids, green vegetables and animal fodder plants of concentrated protein value could be obtained. We have established, too, that the ratio of essential and basal amino acids becomes much more favourable to the purposes of human alimentation, as a result of the live-wilting of isolated leaves, because the accumulation of some essential amino acids during live-wilting is by far superior to that of the basal amino acids (leucine, isoleucine, phenylalanine, valine, methionine, etc.).

In the course of water deficiency, resp. live-wilting essential differences were found between the various plant species in regard to proline increase. During live-wilting in the light, the proline accumulation in the leaves of garden sorrel and spinach does not reach the tenfold amount of the fresh control, while the proline content of the leaves of garden cabbage and sunflower exceeds that of fresh leaves more than a hundred times.

We have established that the increase of total amino acids, as a result of artificial live-wilting under illumination, is of very high degree, ranging from 300 to 600 per cent (in the percentage of control, i. e. of the non-wilted dry matter). Such extremely high accumulation of free amino acids can be achieved only in case of leaves that are well-supplied with mineral nutrients and have considerable carbohydrate and nitrogen reserves, but not in case of the leaves of under-nourished, weakened plants.

The live-wilting processes lasting two-three days in the light have shown that no significant change either in the dry-weight of leaves or in the amount of the soluble total protein, while the total free amino acid and particularly the proline accumulates in high degree (garden sorrel, spinach, garden cabbage, maize, lucerne, sunflower).

As to proteins, a similar statement comes from TVORUS (1970), as well. In our opinion, the cause of the high-level amino acid accumulation is that in case of water deficiency the preponderance of protein synthesis over protein decomposition ceases to be (FARKAS, 1963, 1968; DÉZSI et al., 1970). The growth of cells and cell-walls, as well as cell division are stopped. At the same time, in the leaves the synthesis of protein-building amino acids goes on for a while undisturbedly in fact it is often intensified in the initial period of water deficiency (CHEN et al., 1964; BRITIKOV, 1965; SAVITSKAYA, 1965, 1967; BARNETT and NAYLOR, 1966; STEWARD et al., 1966; DOVE, 1967, etc.) as long as the carbohydrate and N-reserves of leaves are not used up. The plant suffering from water deficiency does not continue to grow. In case of strong

water deficiency, metabolic activity is interrupted between some organs of the intact plant, i. e., the leaves, the stem and root, as well as the growing points (meristems), (BRITIKOV et al., 1965; DOVE, 1967). In such cases the circulation and removal of some water-soluble materials are stopped, among them that of the compounds regulating the metabolism (PÁLFI, 1970). And also the outflow from the leaves of the synthesized organic matters, including amino acids and carbohydrates, stops too (THOMPSON et al., 1966). In case of strong water deficiency the leaves of intact plants also behave like isolated leaves (DOVE, 1967; PÁLFI, 1970).

In case of water stress, the same biochemical processes take place in the leaves of intact plants as in the isolated leaves. The process lasts as long as the plant does not reach a critical physiological state because of water deficiency. A 60–70 per cent water loss of leaves, is already followed by slow necrosis.

If the synthesis of proteins and nucleic acids did not decrease at a time of strong water deficiency (UDVARDY and MÁRIA HORVÁTH, 1964; CHEN et al., 1964; DOVE, 1967; MÁRIA HORVÁTH and LONTAI, 1968; PROCENKO et al., 1968; VLASYUK et al., 1968; TVORUS, 1970, etc.), then we could expect a considerable protein increase instead of the large amount of free amino acid, resp. proline, during the live-wilting of the isolated leaves in the light.

As already demonstrated by STEWART et al. (1966), in case of water deficiency the carbon-chain (α -ketoglutarat) and energy (ATP) for synthesis and accumulation of the amino acids are supplied by the oxidation of sugars. In case of artificial live-wilting it is therefore most important that the isolated leaves have due carbohydrate and nitrogen reserves. From among amino acid, the dicarbon-acids occur in plant proteins only in form of amides (glutamine, asparagine), as stated by STEWART and co-workers. As under the influence of water stress the positive organic matter production, resp. the synthesis of proteins decreases (and therefore the cell division and growth are stopped) there are comparatively more amides discharged from, than incorporated into the proteins. For synthesis and transamination of amino acids the NH_2 -groups are supplied, apart from the inorganic nitrogen, by this amide surplus.

KEMBLE and MACPHERSON (1954) did not consider the wilting of grasses after being cut as a physiological process and regarded the accumulation of free amino acids as the result of simple proteolysis. RADENKOVA (1963), PETINOV (1963), and BRITIKOV et al. (1965), as well as SAVITSKAYA (1965, 1967) described the amino acid resp. proline accumulation in connection with the water loss of leaves as a specific defensive reaction of plants, where the role of proline was interpreted with complex functions. According to STEWART et al. (1966), the presence of reserve carbohydrates is indispensable for the amino acid and proline accumulation in the isolated leaves wilting in the dark.

According to the authors, the inhibitors of the tri-carbon-acid cycle impede the accumulation of proline in case of water stress. These data are supported also by the results of our own experiments concerning the saccharose infiltration before wilting (PÁLFI, 1968a, 1969a, c), as well as by the results of our 2,4-dinitrophenol-infiltration experiments for uncoupling the oxidative phosphorylation (PÁLFI, 1968a, 1969a). As a matter of fact, no proline accumulation takes place either in the light or in the dark in leaves infiltrated by 2,4-DNP.

If prior to live-wilting a considerable amount of proline has already accumulated in leaves, or they have been infiltrated with proline solution the accu-

mulation of proline continues during subsequent live-wilting in the light, in spite of the treatment with 2,4-DNP. It may be supposed that the free proline takes part in the regulation of respiration, as well, by forming NAD-H₂ or NADP-H₂, in case of its transformation into glutamic acid, to the oxidation of which is connected, as a substrate of the terminal oxidation, a great part of the ATP synthesis of the cell (FARKAS, 1968).

BRITIKOV and LINSKENS (1970) infiltrated spinach and maize leaves with proline and found that the normal respiration of tissues is considerably stimulated by proline. Camille HUBAC (1967) infiltrated rape-seedlings with proline and found that drought resistance is vitalized by proline in case of water stress. Similar conclusion were drawn by TYANKOVA (1966, 1969), KÜDREV (1967), and PAVLOV (1969), as well.

During live-wilting of isolated leaves for two days is turned out, that without light both the dry matter and the soluble total protein decreased in the leaves of all the six plants investigated. This fact is also supporting the important role of photosynthesis, resp. of its products, the carbohydrates, in the amino acid metabolism during live-wilting (STEWART et al., 1966; SZÁSZ et al., 1969; MORRIS et al., 1969). In regard to the nitrogen source it might be suggested that the plants under optimum conditions have always at their disposal some potential, reserve nitrogen for a further amino acid and protein synthesis resp. for protoplasm and cell growth. In case of water deficiency this potential nitrogen can still be used at the amino acid synthesis but no further protein increase can be realized any more. The quantity of the rest nitrogen of leaves can be increased by supply of nitrogen through the leaves before being cut off (PÁLFI, 1960) or by nitrogen infiltration after isolation (with a 0,01–0,1 p. c. solution of ammoniumphosphate or -nitrate or urea, as well as 0,5–1,0 p. c. saccharose solution).

The artificial „live-wilting” (bio-dehydration) of isolated leaves and shoots, is under legal defence, as a Hungarian patent. Its title: „Method of procedure for producing plant preparations enriched with free amino acids” (November 26, 1969).

Summary

1. We have named „amino acid metabolism of proline type” the high-degree accumulation (of 200–500 per cent) of free amino acid in leaves that is accompanied by an extraordinary, proline increase of 1000–10 000 p. c. It was found that „amino acid metabolism of proline type” only develops as a result of water deficiency or of physiological drought. „Amino acid metabolism of proline type” is a general regularity quite current in the plant kingdom. The demonstration of proline with an isatine-paper indicator may be used also for characterizing diagnostically the water deficiency of plants, as well as for selecting the drought and frost-resistant plant varieties.

2. The development of „amino acid metabolism of proline type” was equally demonstrated in the course of water loss of isolated leaves in the light and thus the wilting of isolated leaves is also a physiological process. By „live-wilting” we mean the uninterrupted, „increasing water deficiency” of the isolated leaves or shoots, lasting for 2–3 days (bio-dehydration).

3. As a result of the artificial live-wilting, taking place in the light and under optimal conditions, the accumulation of the free total amino acid is, of very high degree, ranging from 500 to 600 per cent, in the percentage of the dry matter of not wilted, i. e. fresh leaves (garden sorrel, spinach, garden cabbage, maize, lucerne, sunflower). During live-wilting in the light for 2–3 days there was no significant change in the dry weight and soluble total protein content of leaves while the free amino acid content of leaves was multiplied. At the same time, in case of isolated leaves, live-wilted in the dark, the amount both of dry matter and of the soluble total protein considerably decreased and the increase of free amino acid was also inferior to that of its light variant.

4. In case of strong water deficiency the leaves of intact plants also behave as isolated leaves. The same biochemical processes take place in case of water stress in the leaves of intact plants, as isolated leaves.

5. In case of water deficiency, the carbon-chain and energy for the synthesis and accumulation of amino acids are supplied by the oxidation of saccharoses (α -ketoglutarat + ATP). It is therefore important that the isolated leaves should have due carbohydrate reserves before live-wilting and that the intensity of photosynthesis should not excessively decrease for 2–3 days, as yet.

6. As a result of water deficiency, comparatively more amides are discharged from the protein than are incorporated since plasm increase and cell division are stopped. For the synthesis of amino acids the NH_2 groups are supplied, apart from the inorganic nitrogen, by this ammonia surplus.

7. During live-wilting in the light there was no considerable increase of proline-content if the leaves were previously infiltrated with 2,4-DNP. The production of proline from carbohydrates takes place by means of the oxidative phosphorylation, uncoupled by 2,4-DNP. If, however, the leaves had contained, already a considerable amount of proline (0,5–1,0 p. c.) prior to 2,4-DNP-treatment, the effect of 2,4-DNP did not manifest itself, and the accumulation of proline continued in the course of live-wilting. It may be supposed that the free proline participates in the regulation of respiration as well, for, if the proline is transformed into glutamic acid, a reducing potency ($\text{NAD} \rightarrow \text{NADH}_2$) is released that can be used for the ATP synthesis.

8. It was found that, with an artificial live-wilting process, as a result of high-level accumulation of amino acids, green vegetables and fodder plants of concentrated protein value can be obtained. We have demonstrated that the ratio of the essential and the basal amino acids becomes much more favourable for human nourishment, as a result of the live-wilting of the isolated leaves.

9. It was found that „amino acid metabolism of proline type” manifests itself in any phase of the development of plants. The largest amount of proline and total amino acid however, is accumulated in the „critical period of water supply”. In case of herbaceous plants this period falls on the time of microsporogenesis.

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