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EFFECT OF THE COLD SOIL AND PHYSIOLOGICAL DRYNESS ON THE AMINO ACID METABOLISM OF WHEAT, BEANS SUNFLOWER, AND PAPRIKA

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Introduction

The physiological bases of the cold — and drought — resistance of plants are little known, as yet. It is already ascertained that the frost-resistance of cereals is connected with the degree of carbohydrate and free amino acid content of the plants (DÉVAY, 1962). From the work of KISS and POZSÁR (1968) it also appears that the increase of the free amino acid level is not a cause of cold-resistance but, owing to the repressed intensity of protein synthesis, as one of its immediate consequences.

Unfavourable external conditions lasting for several days (PÁLFI, 1965, 1968a) or contagious plant diseases (FARKAS, 1963; FARKAS and KIRÁLY, 1961) involve a decrease in the intensity of protein synthesis, and protein decomposition prevails. At that time a considerable amount of free amino acid, as well as glutamine and asparagine accumulate. The increased concentration of free amino acid and amide concentration here and there may be also a consequence of the abundance of nutrition supply (PÁLFI, 1965). According to FARKAS, (1968), an intensive protein synthesis is sometimes accompanied by a high „total amino acid level". It became necessary to look for a biochemical index on the basis of which a correct consequence can be drawn from the increased concentration of amide and amino acid.

In case of a lasting water deficiency the total amino acid and amide concentration of plants rises, as well (PÁLFI, 1968b). It has been supported by several experimental results that at the water deficit of plants the proline content of leaves is multiplied manifold as compared with the control of optimum water supply (BARNETT, 1966; PETINOV and BERKO, 1965; PROTSENKO et al., 1968; STEWART et al., 1966; SAVITSKAYA, 1965; VLASYUK et al., 1968).

In the course of our experiments carried out with twelve mono- and dicotyledonous species belonging to eight families (PÁLFI, 1968b, c) we demonstrated that the several hundred per cent increase of the proline content of leaves occurs exclusively as a consequence of water deficit. We have further ascertained, with culture pot and field investigations that this phenomenon manifests in every phase of development during the life of plants. According to our data, we may conclude the water deficiency of plants from the considerable increase of the proline concentration of leaves. On the basis of this fact, we demonstrated in several plants (PÁLFI and JUHÁSZ, 1968) that a high total salt concentration of water that is present in soil in optimum quantity induces, by increasing the osmotic pressure of the medium, physiological dryness, and a water deficit in agricultural plants.

GOAS (1966) found the proline content of halophytic plants growing in a saline and strongly saline seaside soil as abnormally high. According to GOAS it is doubtless that high salt content of the medium brings about physiological dryness.

During our experiment we are getting on with investigating the influence of the high salt content of the root medium on the amino acid composition of plants. We would like to elucidate, as well, what kind of change is engendered in leaves and water balance of plants concerning the amino acid spectrum by a soil that is colder than optimum. The rising flow of materials produced in the roots and regulating the metabolism of shoots may cease to exist in the time of drought, as well as in isolated leaves. The extremely strong accumulation of proline takes supposedly place owing to the lack of a substance regulating the metabolism in the leaves. In case of isolated leaves, the root-hormone supply similarly comes to an end. It is questionable whether, or not, the great proline concentration appears also at isolated leaves kept in a water saturated state for several days.

We should like to establish, too, if proline accumulates also in the root system of plants as a consequence of its water deficit.

SVEDSKAYA and KRUZHILIN (1966) investigated the influence of cold on a lot of plant species and established that vernalization implies an accumulation of free proline. After cold treatment proline is used — according to them — for protein synthesis during germination as buds become differentiated. TRIONE et al. (1967) demonstrated, as well, a great proline accumulation owing to the cold effect of wheat vernalization. It is questionable if the proline accumulating effect of cold treatment takes only place in the time of germination.

TUMANOV and TRUNOVA (1967) studied the role of sugar accumulation and cooling down in respect of the winter wheat being weather-hardened against frost. According to their results, the fall in temperature itself is not enough for being hardened, also the plentiful supply of sugar to the cells is necessary. This fact is in connection with our experimental result (PÁLFI, 1968b; PÁLFI and JUHÁSZ, 1968) according to which proline accumulation in plants, as a result of water deficit, does not come from a protein but from carbohydrate decomposition.

Materials and Methods

We carried out experiments with wheat „Bezostaya 1", sunflower „from Kisvárda", beans „Black prince", and a red pepper (paprika) sort „57—13".

First we studied the amino acid spectrum of the leaves of plants durably cultured at optimum water supply and those suffering from water deficiency. Then we prepared irrigation-water, contining the salts of Na, K, Ca, Mg chloride and sulfate (van't HOFF's balanced solution with 2 p. c. total salt content and pH value 6, with a composition similar to the extract of saline solis). We already published the exact composition of van't Hoff's sodic salt solution (PÁLFI and JUHÁSZ, 1968).

The culture pots were filled with a mixture of sand and soil (2 : 1). We endeavoured to grow the plants under optimum conditions, in sunshine, from time to time irrigating them with KNOP's nutrient solution as well. The soil of the 30 day old sunflower was irrigated in every second day with running tap water and in the intervening days with van't HOFF's solution of 2 p. c. total salt content. The controls were irrigated, as before, every day with tap water. In this way, both varieties obtained daily the same amount of water. After salinating the medium for a fortnight, we retained leaf samples for analysis. For eight days the salt content of the soil was increased in the same way also in the media of 14-day old wheat, 21-day old beans, and for a fortnight in the medium of 50-day old paprika plants.

For cooling the soil, the paprika, sunflower, bean and wheat plants were grown till they reached the age of 50, 30, 21, and 14 days, respectively similarly in culture-pots filled with sandy soil but under artificial illumination (4000 Lux for 12 hours per day). Then the plants were removed with some soil and transplanted to a 12 cm high glass vessel of 7 cm in diameter. In the next two days the damaged plants were rejected. By that time the air temperature (24 °C) was taken over also by the medium in the pots. Then the pots of one of the varieties were placed into liquid coolant, cooled to 8 °C for 8 hours and incubated under this condition for three days (paprika, sunflower, beans). The root medium of the control remained at 24 °C. The wheat shoots remained on 16 °C and the root medium on 0 °C for four days. At the control plants both shoots and roots were on 16 °C. During the experiment, the soil of the cooled and non-cooled varieties were in the same humidity level (70 p. c. water content calculated for the full water capacity of the soil). In three or four days the plant leaves of were fixed.

The ethanol (50 p. c.) extracts of 200 mg of the leaves dehydrated and pulvenzed were developed on an ascending one and two- dimension paper (butanol-acetic acid-water; 2 to 1 to 1, and phenol-metanol-water; 3 to 1 to 1). The method has been described by SZALAI (1957) and also by us (PÁLFI, 1963) in details. At proline, the blue colour of isatin reaction was measured with spectrophotometer. In determining the total amino acid, the red colour of the copper-salt complex following ninhydrin was measured with the comparative standard mixture method (PÁLFI,1965).

Experimental results

It can be observed in Fig. 1 (stripes A and B) that, as a result of drought, and of water deficit, the total amino acid and amide content of leaves of thc sunflower is considerably higher than in the control with optimum water supply. This amino acid picture is similar to the amino acid composition of infected, diseased plants (PÁLFI and DÉZSI, 1968). It is, however, essentially differing from it in respect of one index: the extremely high proline concentration. It is readily seen from Fig. 1. as well, that in case of applying a phenolic solvent fewer amino acid stains come apart but proline is present in the highest Rf-value.

In plants of lasting optimum water supply — particularly in monocotyledons — the normal proline quantity has been present only in traces throughout the analysis. For multiplying this proline quantity, no quick or high-degree water deficit is needed. A sevaral hundred per cent inctrease of proline concentration is brought about even by a small water deficiency when lasting for number of days, much earlier than the shoots and leaves obviously turn wilted.

In respect of the ion antagonism, the varieties irragated with a balanced van't HOFF's solution of an increased total salt content have considerably been backward in growth than plants irrigated with rainwater. A strong slight appeared for the control (irrigated with rainwater) as regard to size and thickness of leaves, and stems. It appears from Fig. 1 (stripes C and D) that the amino acid picture of the leaves of plants grown in a medium made saline is similar to that of plants suffering from water deficiency. This similarity manifests not only in the greater total amino acid content but also in the greatly increased proline content.

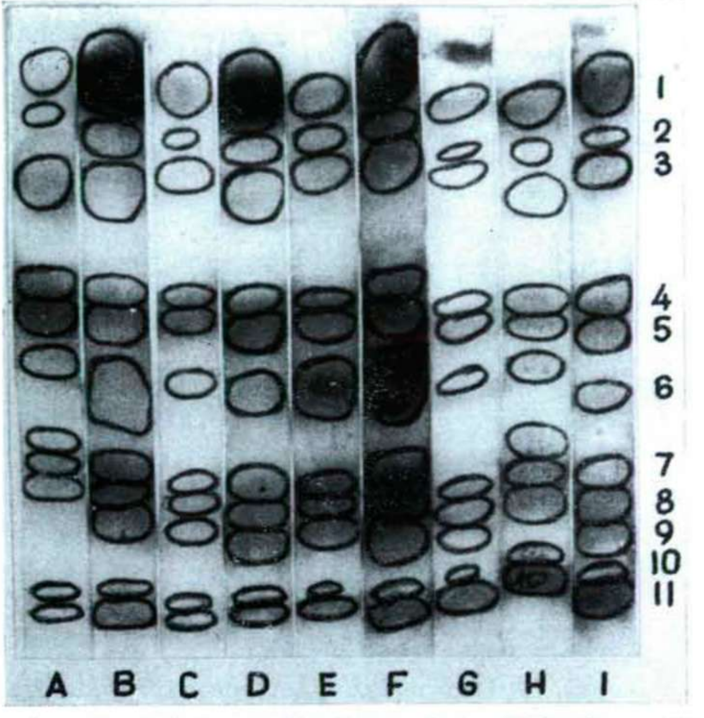

Fig. 1. Free amino acids of the leaves of sunflower plants suffering from water deficit and supplied with water. A = control, irrigated; B = not irrigated, water-dificient; C = control, irrigated with running tap water; D = irrigated with saline-sodic water; E = irrigated; shoots and roots on 24 °C; F = irrigated, shoots at 24, roots at 8 °C; G, H, I = comparative standard with 25, 50 and 100 μg total amino acid content. 1 = pro; 2 = Phe; 3 = Leu; 4 = Val+Met; 5 = γ-Amb; 6 = Glu—NH_2+Ala+Arg; 7 = Thr+Lys; 8 = Asp—NH_2+Gly; 9 = Glu+Ser; 10 = Cys; 11= Asp.

It is known that in the natural saline soils, too, Na-salts are dominant like in our van't HOFF's irrigating solution. In plants grown in these hind soils a large amount of Na^+-ions may accumulate, competing with other nutrient ions during uptake (PÁLFI, 1963). When measuring the Na-content of leaves with flamephotometer it appeared that the Na-concentration of leaves of the variety grown in a medium of increased salt content became 20 times higher than that of a control irrigated with tap water, what is in fact, a damaging factor (LAPINA, 1967).

In spring, in cereals often a major difference occurs between the temperatures of the root medium and of the air. Under such circumstances a dry wind may quickly spring up that is relatively warm as compared to the temperature of soil. Thus, the transpiration of shoots increases and at the same time in the cold soil the vital functions of roots decrease.

Figure 1 is showing (stripes E and F) that an accumulation of amino acids and particularly of proline is engendered also by a temperature (8 °C) that is very low as compared with that of the air (24 °C) and lower than optimum; like in the case of water deficient plants (stripe B) or high salt content of the soil solution (stipe D).

In Fig. 2, we are demonstrating the amino acids of the leaves of paprika, sunflower, and bean plants the roots of which were cultured in a cooled (8 °C) or non- cooled (24 °C) soil and their shoots in an temperate (24 °C) air. The three stripes (C, E, and G) of the figure contain extremely large and dark stains, the same stripes which are containing the extracts of leaves of the plants cultured cooled soil. The total amino acid and proline concentration of the leaves of plants cultured cold soil is therefore considerably higher than

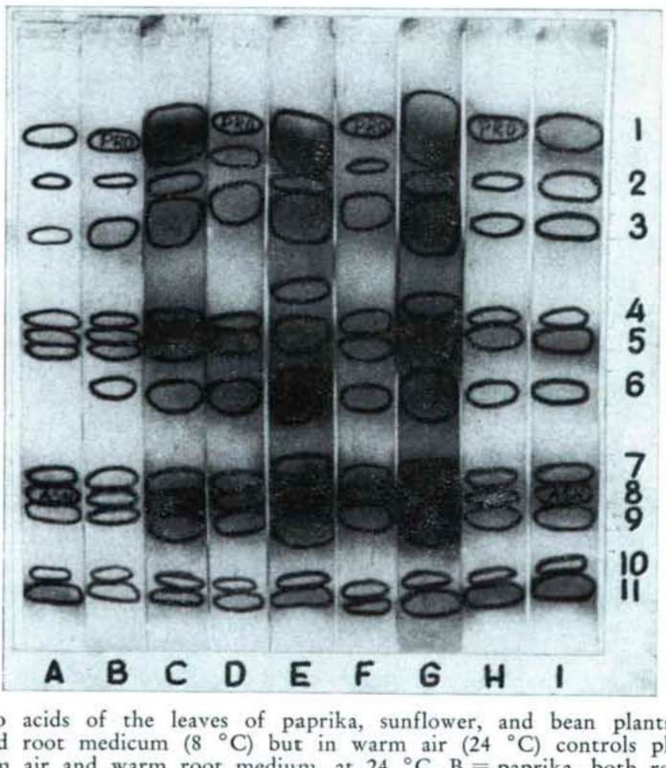

Fig. 2. Amino acids of the leaves of paprika, sunflower, and bean plants cultured in a cooled root medicum (8 °C) but in warm air (24 °C) controls plants cultured in warm air and warm root medium, at 24 °C. B = paprika, both root and shoot are warm (24 °C); C = paprika, the root at 8 °C, shoot at 24 °C; D = sunflower, both root and shoot are warm; E = sunflower, the root at 8 °C, shoot at 24 °C; F = beans, both root and shoot are warm; G = beans, root at 8 °C, shoot at 24 °C; A, H, I = comparative standard, proline is 2,5 and 5, resp. 10 μg. 1—11 = The same as in Fig. 1.

that of varieties cultured warm soil (stripes B, D, F). This phenomenon may be rather common for we have carried out our experiments on several species of cultured plants.

We performed our experiments with cooling the rootmedia of thermophilous plants germinated under comparatively high temperature. Now we turn to the results of our investigations in cooling concerning the winter wheat „Bezostaya 1", a comparatively cold-ressistant plant species. In what we cooled the root medium to a even lower temperature (0 °C) and the air temperature was lower (16 °C), too. We continued cooling for four days.

Fig. 3. Wilted wheat plants cultured in cold root medium (0 °C) but in warm air (16 °C) (on the riht) and control on the left — turgescent plants cultured in warm root medium and warm air.

Figure 3 demonstrates that the longer shoots cultured in cooled root medium have fully lost their turgor and bent down wilted. It is noticeable, that the water content both of the soil of 16° and that of 0 °C was equal. The cooled roots were, therefore, in capable supplying the shoots with water. The fresh weight of shoots of this variety was 32 p. c. smaller than that of the non-cooled variety. It is interesting that in the dry weight of shoots we have not found any difference between the two kinds of treatment. In quantity and dry weight of the roots however, a considerable difference appeared. The dry matter of the cooled roots was 22 p. c. smaller than that of the variety cultured warm. For comparison, the leaf extracts of wheat plants suffering from water deficit owing to drought and irrigated with water sendered saline, as well as those cultured in a cooled medium were put on the stripes of a chromatogram paper (Fig. 4).

It appears from Figure 4 that the shoots of plants containing an optimum quantity of water but cultured in a soil of high soluble total salt content

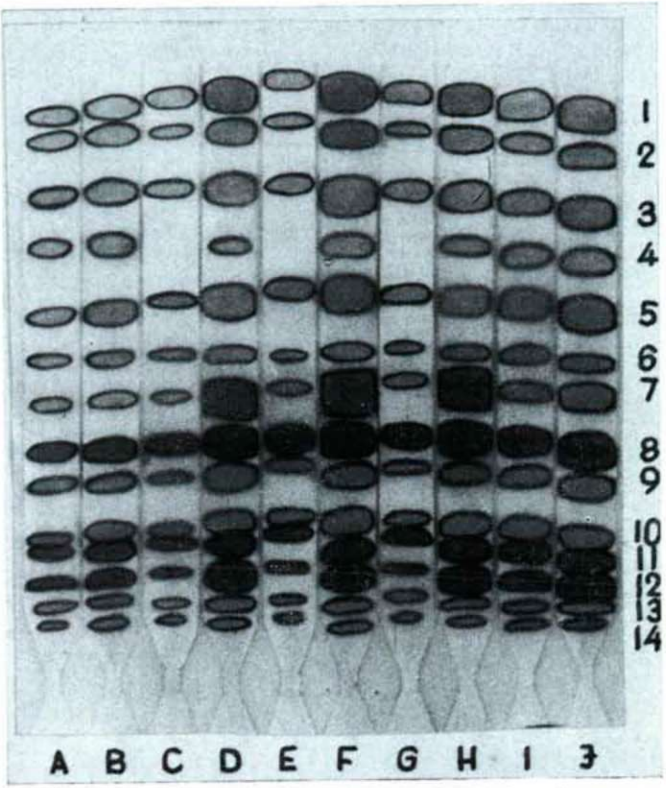

Fig. 4. Amino acids of the leaves of wheat plants suffering from water deficit and with optimum water supply. C = control, irrigated; D = not irrigated, water-deficient; E = control, irrigated with running tap water; F = irrigated with saline sodic water; G = both shoot and roots at 24 °C, irrigated; H = shoot at 16 °C, roots at 0 °C, irrigated; A, B, I, J = comparative standard, order of proline: 2,5, 5,0, 7,5 and 10 μg. 1 = Leu; 2 = Phe; 3 = Val+Met; 4 = pipecolic acid; 5 = γ—Amb; 6 = Tyr; 7 = Pro; 8 = Ala; 9 = Glu+Thr; 10 = Gly+Ser; 11 = Glu—NH_2+Ser; 12 = Asp—NH_2+Arg; 13 = Lys+His; 14 = Cys.

(stripe F) or of low temperature (stripe H) contain an extremely large quantity of proline (32 and 25 μg) just like the plants suffering from water deficit owing to the low water content of the soil (stripe D: 22 μg). The controls of the same plants (stripes C, E, and G), i. e. the leaves of plants cultured in a non-cooled soil, irrigated with the optimum quantity of water with small salt content are wqually containing a very low quantity of proline (2. 5 μg). In Figure 1, Table II, the amino acid stains of standards of a measured amount (stripes A, b, I, J) enable us to carry out a rough quantitative estimation.

We should also mention that the chromatogram of Fig. 4, was made, distinguishing is from the former ones, in a butanol solvent; its development was slowed down by cooling. In case of tempering like this, though occurring rarely a non-protein forming amino acid, the pipecolic acid may appear as independent statins. It is appearent in Fig. 4. that, besides the standard stripes, also the leaves of varieties cultured under unfavourable external conditions (stripes D, F, and H) contain pipecolic acid. Pipecolic acid (PÁLFI, 1965, 1968a; PÁLFI and DÉZSI, 1968) is a sing of a weakened physiological state. It can generally be demonstrated only when in the leaves protein synthesis diminishes and protein decomposition prevails.

In the one-dimension chromatograms some amino acids do not come apart from other amino acids but form with them a common, complex stain. As in case of a two-dimension development a better separation can be obtained, to we which publish data, about wheat cultured in non-cooled and cooled media (Fig. 5). It should be mentioned that, as we have tried to get a clear picture about amino acids, we have prepared one- and two-dimension layer- and paper-chromatograms, too, in several repetitions from every extract of every investigated plant.

In Figure 5. becomes immediately obvious that the stains of amino acids are considerably larger in the chromatogram of wheat cultured in a cold root medium (O °C) than in the non-cooled variety. In the varieties cultured in low temperature very large glutamine and asparagine and particularly large proline stains are seen. In this variety pipecolic acid appeared, indicative of an intensive protein decompensation.

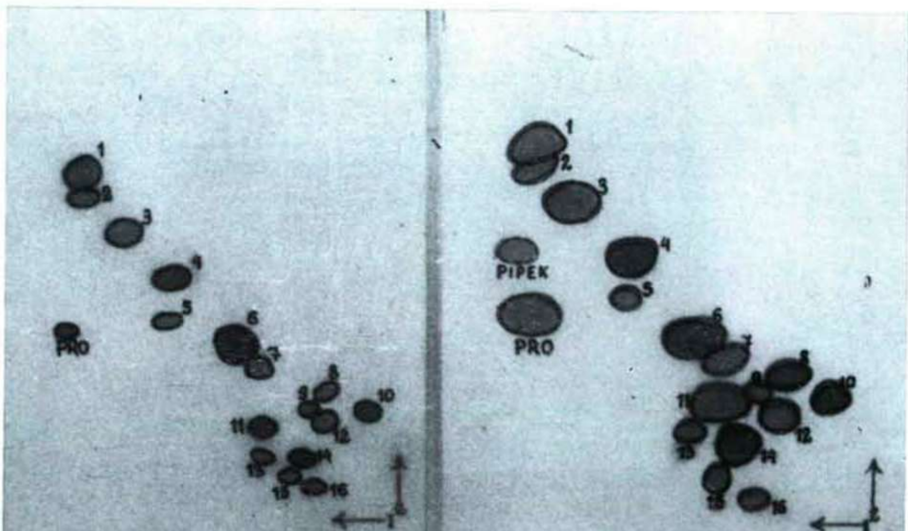

Fig. 5. Amino acids of the leaves of wheat plants cultured in a cold root medium (0 °C) but in warm air (16 °C) (on the right and their control) on the left — wheat plants cultured in warm root medium and warm air, i. e. at 16° C). Layer chromatogram. 1 = Leu; 2 = Phe; 3 = Val+Met; 4 = γ—Amb; 5 = Tyr; 6 = Ala; 7 = Thr; 8 = Glu; 9 = Gly; 10 = Asp; 11 = Glu—NH_2; 12 = Ser; 13 = Arg; 14 = Asp—NH_2; 15 = Lys; 16 = Cys.

In the following we are trying to elucidate whether the several prolines of isolated leaves really appear as a consequence of water deficit and not as a lack of the root factor regulating leaf metabolism. For that purpose we have had the sunflower and bean leaves excised in a water-saturated state for four days. During treatment the leaves were immersed in water twice a day for four-four hours, in the eight-eight hours intervals we incubated them under a wet filter-paper in big PETRI dishes under humid condition. In the meantime, during the immersions in water, and also for further six hours a day, they were illuminated.

Stripes B, C, D, and E of Fig. 6. demonstrate the free amino acid content of the isolated leaves of sunflower and beans cultured in a water-saturated

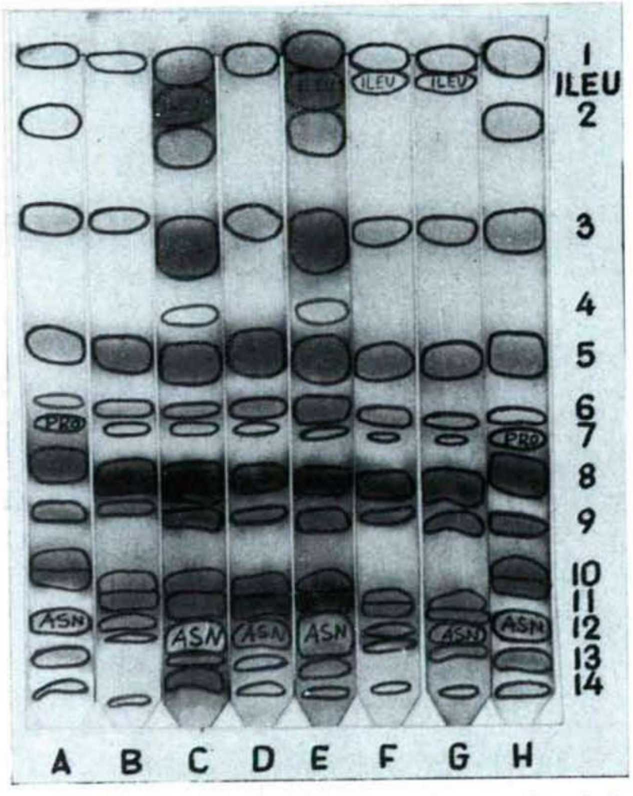

Fig. 6. Amino acids of isolated sunflower and bean leaves, cultured in water-saturated state for four days and the controls, as well as amino acids of the roots of irrigated and water-deficient wheat plants. B = sunflower leaves, immediately fixed after excision (control); C = sunflower leaves, incubated water-saturated for four days after excision; D = bean leaves, immediately fixed after excision (control); E = bean leaves, incubated water-saturated for four days after excision F = water-supplied (irrigated) wheat root; G = water-deficient wheat root (not irrigated); A, H = comparative standard, with 75 and 100 μg total amino acid content.

state for four days is considerably higher than that of leaves fixed immediately after excision. Isoleucine, phenylalanine, valine + metionine, and asparagine have particularly large stains. In the isolated leaves pipecolic acid has appeared, as well. The proline concentration of isolated leaves incubated for four days, is, however, quite low and does not surpass that of controls.

From Fig. 6 it is readily concieved that the proline content of the wheat root suffering from water deifcit (stripe G) is not higher than that of wheat with an optimum water supply (stripe F). It may also be established that the proline content of wheat root is very low.

Evaluation of results

Proline occurs among the free amino acids of the leaves of plants cultured under optimum conditions but in a very small amount. If, however, the plants have suffered from water deficit for two-three days, the proline content of leaves becomes a multiple of the normal amount. We already demonstrated (PÁLFI, 1968b; PÁLFI and JUHÁSZ, 1968) that the increase of many hundred per cent of the proline content of leaves is caused solely by water deficit. On the basis of our data, therefore, a conclusion can be drawn from the considerable increase in the proline concentration of leaves to the water deficit of plants.

The data obtained in the course of investigations carried out on sunflower, wheat, bean and paprika plants umanbiguosly demonstrate that the plants can suffer from water deficit even in case of an optimum amount of water in the soil with a high total salt content. The water deficit of plants cultured in a soil solution with high salt content was indicated by the extremely high proline concentration of leaves. It was proved, as well, that in the leaves of plants cultured saline medium the Na-amount increases twenty times as high as that of the control irrigated with tap water, what similarly causes damage (LAPINA, 1967; PÁLFI, 1963).

The proline may have, suggested also by other researchers (PROTSENKO et al., 1968; VLASYUK et al., 1968), in case of drought a role of increasing the hydration of protoplasm. In addition, it can serve as an auxiliary matter storing nitrogen, transformed into glutamic acid under favourable conditions (STEWART et al., 1966).

Water deficit may result too, if the roots of plant live in a soil of low temperature and its shoots in a streaming dry air of a considerably higher temperature. In the cold soil the activity of roots concerning water supply and transport decreases and the roots do not supply, water therefore, water deficit occurs because of strongly transpiring shoots. In our experiment we have endeavoured to clear up whether a physiological dryness like that is indicated, too, by the strong increase in proline concentration. It is proved by the results obtained on paprika, sunflower, bean and wheat that the proline concentration of leaves becomes manifold also as a result of water deficit caused by the low soil temperature. This water deficit may occur not only in the case, of a decreased water content of root medium but also when a proper quantity of the soil solution is available for the plants but, owing to their high osmotic pressure or very low temperature, they need a higher energy consumption (physiological dryness).

We have already demonstrated that in the case of water deficit the great amount of proline is not directly the issue of protein decomposition (PÁLFI, 1968c). It became known that the isolated leaves which are slowly wilting in darkness synthetize proline until their carbohydrate reserve is exhausted. After the sugars have been used up, the great amount of proline produced till then also decomposes before the leaves wither. By saccharose infiltration of cut off leaves wilting in darkness we have proved that the high quantity of proline in plants suffering from water deficit had been caused by sugar (PÁLFI, 1968c; PÁLFI and JUHÁSZ, 1968). In the leaves infiltrated with saccharose more proline, was formed and for a longer time than in the control leaves infiltrated by water.

For hardening the winter wheat against frost it is indipensable to supply the cells with plenty of sugar (DÉVAY, 1962; TUMANOV and TRUNOVA, 1967). At the same time, as it has been ascertained, if there is more sugar in the leaves, consequently, more proline is produced as a result of cold. From this connection it may be supposed that proline may also play a role in the frost-resistance.

During our experiment, the proline content of the isolated leaves incubated in light in a water-saturated state did not increase for four days as compared with that of the leaves fixed immediately after excision. These leaves are, of course, not wilted for no water deficit occurred. This fact proves that the high proline concentration of the excised, wilting leaves is not a result of a matter produced in the root — and transported under normal conditions to the shoot — for regulating metabolism but it was caused by water deficit.

We have investigated also the composition of amino acid in the roots of the water-deficient wheat, sunflower, paprika, and beans, ascertaining that, owing to water deficit, the amount of proline does not increase in the roots, therefore it is not transported from leaves to roots. A high degree synthesis of proline realizes only in organs containing chlorophyll. In this phenomenon, the formation of chlorophyll may have some indirect role.

DURANTON and MAILLE (1961) placed proline C^{14} in the leaves of *Solanum melongena* L. After six hours they demonstrated that 1 per cent of the absorbed proline was used up to the process of chlorophyll synthesis. The authors suppose a close connection exirting between the proline metabolism and the biosynthesis of the chlorophyll. We may also suppose that owing to drought the proline synthesis increases and the formation of chlorophyll ceases to function at the same time. COCUCCI and MARRE (1966) investigated aging isolated potato leaves. According to their data, sugars inhibited leaf dises from aging by slowing down the decomposition of chlorophyll and protein. The illumination of dises resulted also in the delaying of aging. The authors suppose that light exerts its effect through the products of the photosynthesis (partly through sugars).

Summary

We have established that the free amino acid and amide content of intact plants and isolated leaves suffering from water deficit increases in a considerable degree. This increase is a result of the stagnation of protein

synthesis. We generally obtain a similar picture for amino acid of infected or diseased plants. The water-dificient plants however, differ from the amino acid composition of diseased plants in an essential character: in the extremely high degree of proline concentration.

We have discovered that the enormous accumulation of proline in the leaves is not the consequence of a lacking root factor in regulating the metabolism of shoots. In case of a considerable water deficit of the plants the streaming of regulating substances from roots to shoots ceases to function. In excised leaves, kept in a water-saturated state for several days, the supply of root factor was though interrupted; never the less, the proline concentration remained on a normal level. We have established that from the raising of the proline content of leaves from the normal level to manifold the only reasonable conclusion is a water deficit of the leaves.

The water deficit of leaves is demonstrated by a considerable accumulation of proline, as an indicator, even if a sufficient amount of water is contained in the root medium, but its uptake by the roots is inhibited or rendered more difficult. Such physiological dryness was engendered by raising the salt content of the water used for irrigation. The considerable increase of the proline concentration in the leaves was already indicated in 2—3 days.

Water deficit developed also if the root system of the plants living in a soil containing an optimum amount of water but of a low temperature, and the shoots were in a dry air of a considerably higher temperature. It is proved by the data obtained in sunflower, paprika, bean, and wheat plants that the free proline concentration of leaves considerably increases also as a result of a physiological dryness like this.

We have ascertained that as a result of water deficit or physiological dryness in the roots of plants, the proline concentration is not superior to the root size of the plants of optimum water supply. A very high increase in the proline concentration, as a consequence of water deficit, occurs therefore, only in the parts of plants that contain chlorophill.

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