CHANGE OF PIGMENT CONTENT, PROTEIN CONTENT AND THAT OF THE RIBONUCLEASE ENZYME ACTIVITY IN INTACT PLANTS AND ISOLATED BARLEY LEAVES

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Introduction

In our experiments for studying the biologic role of roots, we have treated six days old intact MFB barley plants and leaves, detached from their roots, with running water, 10^{-2}M KCN, 2.10^{-6}M actinomycin D, and with a solution containing both inhibitors of metabolism together.

The days of treatment having advanced, the processes of decomposition became more and more conspicuous, manifested in a decrease of pigment content, and in an increase of the amount of the acid-dissolvable nitrogenous fractions and of the nucleic-acid fractions of little polymerisation. The activity of the ribonuclease enzyme has increased.

As regards the study of the functioning of a root system in the life of a plant, and its role in the processes of metabolism, it is an accepted method to register the metabolic changes taking place in the leaves detached from their roots. In a short time after the roots being removed, the decrease of pigment content and protein level of leaves can be observed (Chibnall-Wiltshire, 1954). This decrease is the most accentuated as regards the amount of chlorophyll-a, in connection with a decomposition of the protein complex (Horváth-Lásztity, 1967). The intensity of breathing is increasing (James, 1953) and a shifting of the balance of metabolic processes towards a decomposition can be observed. In the tissue extract of the leaves detached the oxidized NADP is present in a larger amount than in the leaves of intact leaves. Removing the roots is connected with an increased NADPH oxidization that refers to an important role of roots in regulating the respiratory tracts (Horváth-Udvardy, 1965). In the leaves deprived of their roots the amount of free amino-acid, of ammonia (Chibnall-Wiltshire, 1954) and, as a rule, that of nitrogenous compounds soluble in alcohol increases, and the whole RNA level decreases, while the DNA and the total phosphorus, *resp.* the total nitrogene content remain

unchanged (Martos, 1959). In the isolated leaves, the activity of the dehydrogenases of the pentose-phosphatic cycle (Udvardy-Horváth, 1964) and the ribonucleic activity increase (Bagi-Farkas, 1967; Lewis, 1967; Udvardy-Farkas, 1967). Some authors find similar to each other the metabolic changes produced by the roots removed, dried (Lewis, 1967) of infected, and the effect of root-function inhibitors — *e.g.*, tripoflavin. The roots being removed, the metabolic processes become, therefore, unbalanced showing that the root system is not only a site of the supply of materials but also that of other metabolic processes. We can, however, not succeed in clearing up these processes by removing the roots alone, because in the leaves damaged in that way there appear, apart from metabolic disturbances produced by the interruption of root function, other processes, as well, with a tendency of regenerating the root system, i.e., the disturbances caused by the damage (Horváth-Kovács, 1968). And the chemical inhibition of root function of the intact plants is raisling the question if the metabolic changes produced in the leaves by these materials are taking place only as an inhibition of some processes in the roots or the inhibitors, getting to the leaf, have their influence there, as well.

On the basis of the problems mentioned above, we have performed our investigations to observe the metabolic changes produced in the leaves by removing the root and applying some inhibitors.

Material and method

Our investigations were carried out with an MFB sort of barley. The plants were grown in washed river sand, treated with culture fluid, in an artificial plant growing place (Horváth-Lásztity, 1966) and in a greenhouse.

The six days old intact plants of rinsed roots, *resp.* the leaves detached were treated by being placed into tap water, 10^{-2}M KCN, 2.10^{-6}M Actonomycin D, and a solution containing together 10^{-2}M KCN and 2.10^{-6}M Actinomycin D. The concentration of the inhibitors used was selected out of a concentration series that was set previously. Plants from the same sowing and grown in sand for a longer time have been examined as a control.

The investigations were carried out from the first hour after treatment till the ninth day, in five repetitions. The determinations were performed on leaves of 1 g fresh weight. The parts damaged as a consequence of treatment were removed in every case.

The course of the determination of pigment was published in some previous articles of ours (Horváth-Lásztity, 1965).

The activity of ribonuclease enzyme was determined on the basis of the increase of fractions of little polymerisation of yeast soluble in RNA hydrochloric alcohol, demonstrated as a result of the enzyme, and on the basis of the UV absorphtion change, taking place on 260 mμ. The extinction increase of 0.01 as compared to the control is one enzyme unit (Tuve-Anfinsen, 1960; Venetianer, 1964; Dévay, 1965).

The determination of the nitrogenous compound soluble in trichloroacetic-acid of ten percent and that of the protein content were carried out according to Nessler, after being damaged by sulphuric acid.

The semi-quantitative determination of the nucleic-acid fractions of little polymerisation was carried out, after being solved in hydrochloric alchol, with a measurement of UV absorption on 260 mμ. The amount of the nucleic-acid fractions of large polymerisation was counted on the basis of the UV absorption of a fraction hydrolized with 0,3 M KOH for 18 hours in 37 C° and indissoluble in acidifeus alcohol, measured on 260 mμ. A standard curve has been made of yeast RNA (Tankó, 1958).

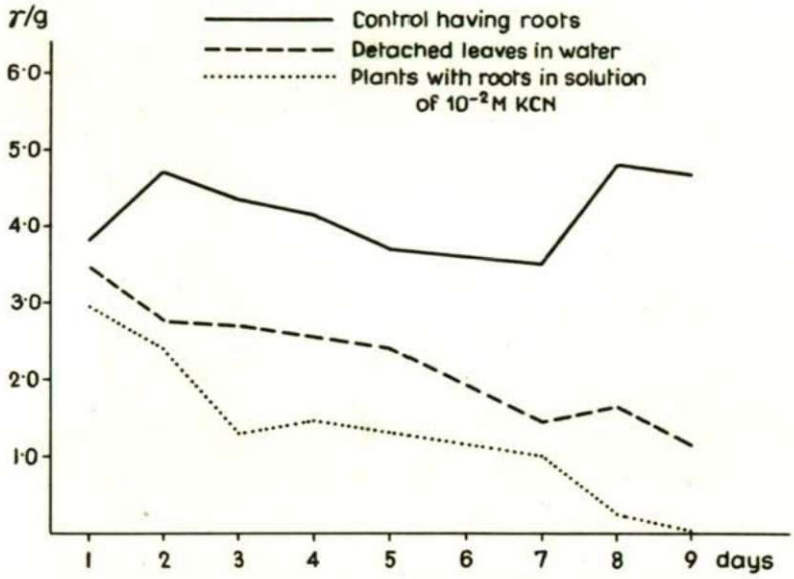

Fig. 1. Formation of the total pigment content, as a result of diferent treatments (γ/g) fresh weight)

Experimental results

In the course of our investigations we have demonstrated that both in the leaves of plants detached and put into water, and in those of intact plants treated with 10^{-2}M KCN, the total pigment content has decreased as a function of time and as compared to the control (Fig. 1).

In the leaves of control plants, in the total pigment content referred to the protein unit, the continuous increase can be demonstrated in the first nine days of treatment while in the leaves detached it does not differ essentially from the value measured in the first day during the same period. On the other hand, in the leaves of plants having roots and treater with 10^{-2}M KCN, the total pigment content referred to the protein unit is decreasing rapidly from the first day of isolation (Table I). The amonunt of chlorophyl-a and b has decreased from 29 p.c. to 11 p.c. and in the leaves of intact plants treated with a cyanide from 29 p.c. to zero, during the period of treatment while, in the same time, it increased from being one-third part of the total pigmet content to be half a part of it in the leaves of control plants (Table I).

Table I. Formation of the pigment content in barley leaves, as a result of a treatment.

Duration of treatment in days	Total pigment content (protein ratio) / pigment (mg protein)			Amount of Chlorophyl a+b in percentage of the total pigment content		
	Control having roots	Detached leaves in water	Plants with roots in a solution of 10^{-2}M KCN	Control having roots	Detached leaves in water	Plants with roots in a solution of 10^{-2}M KCN
1	0,23	0,27	0,18	32	29	29
2	0,44	0,21	0,15	34	33	30
3	0,45	0,23	0,07	38	34	27
4	0,47	0,33	0,07	46	44	27
5	0,71	0,31	0,06	44	34	25
7	0,57	0,25	0,09	52	26	10
8	0,48	0,29	0,03	46	18	7
9	0,73	0,24	0,00	51	11	0

Being influenced by the treatment, the quantity of the acid-soluble nitrogenaus fraction has increased, as demonstrated by the value of these fractions reckoned over into protein units (Table II).

The amount of nucleic-acid fractions of little polymerisation considerably increases in the leaves treated, as compared to the controls (Fig. 2). The increase of the nucleic-acid fractions of little polymeri-

Table II. Formation of the ratio of protein content (mg) of soluble nitrogenous fractions (mg) in barley leaves, as a result of a treatment

Duration of treatment in days	Control with roots	Detached leaves in water	Plants with roots in a solution of 10^{-2}M KCN
1	0,05	0,09	0,09
2	0,09	0,13	0,16
3	0,08	0,17	0,22
4	0,09	0,28	0,14
5	0,11	0,17	0,14
7	0,07	0,24	0,30
8	0,09	0,42	0,33
9	0,12	0,60	0,20

sation is not followed by an increase of the fractions of large polymerisation, as demonstrated by the formation of proportion of these two fractions (Table III).

Table III. Formation of the ratio of nucleic acid fractions of large polymerisation (mg) and those of little polymerisation (mg) as a result of treatment

Duration of treatment in days	Control having roots	Detached leaves in water	Plants with roots in a solution of 10^{-2}M KCN
1	1,7	2,3	2,0
2	1,9	2,7	2,3
3	2,0	3,2	4,5
4	2,0	3,0	2,8
5	1,5	2,5	2,4
7	1,7	2,3	2,5
8	2,3	3,3	3,8
9	2,8	3,6	5,3

Table IV. Formation of the enzyme activity of ribonuclease in barley leaves, as a result of treatment (enzyme-unit/g fresh weight)

Duration of treatment in hours	Control having roots	Detached leaves in water	Plants with roots in a solution of 10^{-2}M KCN
1	1365	1190	1470
3	1190	1260	1085
6	1505	1330	1960
8	1155	1260	1680
12	1260	1155	1575
16	1260	1645	2240
24	1260	1645	3115

The activity of ribonuclease enzyme has increased essentially, as compared to the control, in the leaves detached and put into some water from a tap twelve hours after the isolation. This increase may be observed in the leaves of intact plants treated with a cyanide as soon as after two hours (Table IV). The considerable activity could be observed during our investigations even on the ninth day of isolation, both as a consequence of being detached and treated with a cyanide. The highest activity values were measured, in every case, in the leaves of plants treated with a cyanide (Table V).

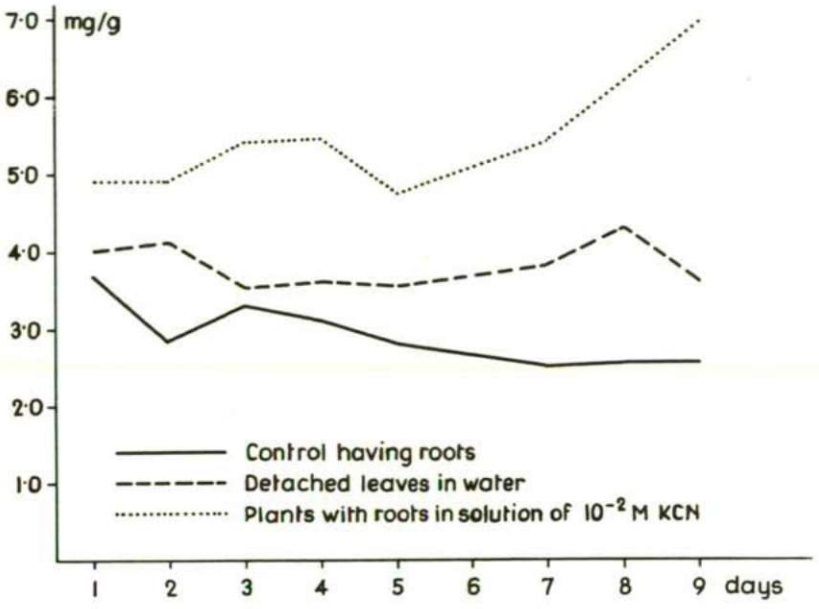

Fig. 2. Foramtion of the quantity of nucleic-acid fractions of little polymerisation — soluble in acidic alcohol — as a result of different treatments (mg/g fresh weight)

Table V. Formation of the enzyme activity of ribonuclease as a result of treatment, referred to a unit quantity of protein (Enzyme unit/mg protein)

Duration of treatment in days	Control having roots	Detached leaves in water	Plants with roots in a solution of 10^{-2}M KCN
1	100,2	121,7	230,4
2	109,1	130,2	258,8
3	135,8	122,4	299,9
4	153,0	191,3	274,6
5	203,4	224,1	324,6
7	165,3	287,3	757,4
8	126,0	377,5	942,5
9	136,5	230,6	513,3

Table VI is demonstrating the influence of the treatment with 10^{-2}M KCN in tap water, with 2.16^{-6}M Actinomycin D, resp. with the

joint application of the two latter metabolic inhibitors, upon the protein content of leaves and the development of the ribonuclease enzyme activity in intact plants and in leaves detached.

Table VI. Formation of protein content and ribonuclease enzyme content, as a result of different treatments, in barley leaves detached and in those having roots. In this experimental series the plants were grown in greenhouse.

Duration of treatment in days	Plants having roots					Leaves detached			
	In sand (control)	In water	In 10^{-2}M KCN	In 10^{-6}M Actinomycin D	In 10^{-2}M KCN + 2.10^{-6}M Act. D	In water	In 10^{-2}M KCN	In 2.1^{-6}M Actinomycin D	In 10^{-2}M KCN + 2.10^{-6}M Act. D
	Protein content/mg protein/g fresh weight								
1	34,38	27,50	44,53	25,47	20,78	28,91	37,03	28,13	23,28
4	26,41	16,09	18,59	16,41	17,34	20,94	20,31	10,78	10,94
	Ribonuclease activity (enzyme unit/mg protein)								
1	37,6	82,7	75,4	74,2	215,5	55,6	92,6	59,7	372,8
4	58,3	139,2	161,7	145,0	316,9	191,9	449,6	172,0	946,9

According to our results, the protein content decreases in every case till the fourth day of treatment. As a result of a treatment with cayanides, both in the leaves of the plants having roots and in the leaves detached, the protein content is higher after a treatment of one day than it is in the control; on the fourth day of treatment, however, even that declined under the value of control. As a result of all the other treatments applied, the decrease of protein content as compared to the control could be demonstrated as soon as after the first day. This decrease is the most expressed as a result of Actinomycin D.

The enzyme activity of ribonuclease increases in every case till the fourth day of treatment. From the different treatments, the joint application of Actinomycin D and KCN shows a conspicuous increase in activity as compared to the untreated control as soon as after the first day. This increase is even more expressed on the fourth day of treatment, giving the highest value of all variations both in the leaves of intact plants and in leaves detached, on the fourth day of treatment (Table VI).

Discussion

According to our experimental results, in the leaves of a six days old barley plant, treated as having roots on being detached, essential differences can be demonstrated as to the development of some characteristics of metabolic processes investigated by us and compared to a control, already after a treatment of one day.

The prevalence of disintegrating processes is shown by the decrease of pigment content as a result of treatment, and by the increase of the amount of the nitrogenous fractions dissolvable in acid and of the

nucleic-acid fractions of little polymerisation, as well by that of the enzyme activity of ribonuclease.

The change of pigment content is first of all shown by the destruction of chlorophyl-a and b. The beginning of a disintegration of green pigments precedes the decrease of protein content, and a full decomposition takes place, as a result of cyanide, as early as on the ninthday of treatment.

According to our results it seems so that the disintegrating processes occur more intensively as a result of cyanide than as a result of the roots being mechanically removed. This observation is supposed also by a change of the enzyme activity of ribonuclease. The enzyme activity increases if damages intensify, being more expressed if influenced by a cyanide than by the roots detached. Therefore, achange in the enzyme activity of ribonuclease is a sensitive indicator of factors influencing the nucleic-acid metabolism, occurring, as demonstrated by Lewis (1967), not only as a result of the removal of roots and of the inhibitors but for instance also as a consequence of a possible scarcity of water.

In the leaves of control plants, as well, an increase of the ribonuclease activity can be demonstrated, on a small scale, in the process of growing old, accompanying a small decrease of protein content. Its cause may have been that the growth of the first leaf had been completed. The activity of ribonuclease is higher in case of every treatment than in the control with roots, and the increase in activity is more and more as the time of treatment is progressing. The increase of enzyme activity is accompanied by a growth of protein content only in case of cyanide treatment, after being treated for one day. In the same variations after a treatment of four days and as a result of every other treatment, the ribonuclease activity increasing more and more may be observed with a decreasing protein content. The ribonuclease activity compared to a control was increased on a small scale by Actinomycin D, being used as an inhibitor of protein synthesis, on the fourth day of treatment, a value elicited by a cyanide could, however, not be obtained. The greatest activities measured were obtained by a joint application of both metabolic inhibitors.

As a result of potassium cyanide, after a treatment of one day the protein content is higher than in leaves untreated; on the fourth day of treatment, however, it is already lower than the control value. Actinomycin D applied separately and together with KCN has induced a decrease of protein content. The enzyme activity of ribonuclease increased in every case as compared to the control, quite apart from the protein content increasing or decreasing.

From the different degrees of the efficiency of inhibitors — manifested *e.g.* in the different activities of ribonuclease — and from the e comparative difference of the metabolic indicators of intact plants, *resp.* of leaves detached the conclusion can be drawn that the influences of the two metabolic inhibitors applied for increasing destruction are controlled by the root system in different ways of metabolism.

Our results are reasing the question af a necessary application of further metabolic inhibitors and of the investigation of the activity of other enzymes for clearing up the problem of control of mechanism.

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