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RELATIONSHIP BETWEEN THE PLANT GROWTH REGULATION AND PHOSPHORYLATION PROCESSES I. EXAMINATION OF THE ALCOHOL SOLUBLE PHOSPHATE COMPOUNDS OF PEA SEEDLINGS BY RADIOPAPER-CHROMATOGRAPHY, APPLYING DIFFERENT LIGHT CONDITIONS AND DIFFERENT INCUBATION TIMES

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Introduction

In studying the effect of plant growth regulators the examination of the relationship between growth and oxidative phosphorylation processes is very significant.

It is known that the most plant regulators are also active as regulators of the oxidative phosphorylation. Many authors believe the basic reaction of the effect of plant hormones and of other regulators begins by the influence of phosphorylation reactions (Marinos and Hemberg, 1960; Sen-Gupta and Sen, 1961; Flaig and Schmid, 1962; Mc Daniel and Sarkissian, 1966).

According to the fact that the satisfaction of the energy requirement is most important for the growth the view of the above mentioned authors can be real, but the results of other authors (Stenlid and Saddik, 1962; Spring and Rowan, 1966) don't allow the generalisation of this rule.

For the determination of the oxidative phosphorylation there is an in vitro laboratory method by the measurement of the P/O quotient. The open question is whether the „in vivo" processes are similar to the results of in vitro measurement or not; the question is therefore whether changing the P/O quotient by plant regulators is the same in the plant tissues and in vitro or not.

The difficulty of in vivo experiments is that to study the regulator effects is possible only by indirect methods on the basis of determination of the ratios of the inorganic phosphates and of the high energy phosphate compounds.

The classical analytical methods being less effective for this determination, the authors worked out an in vivo method, to find the suitable

conditions for the experiments. They separated the inorganic phosphates and the different low and high energy phosphate ester compounds by radiopaperchromatography. The amount of them and their ratios was measured by their activities.

In the first step the seedlings were kept in nutrient solution containing ^{32}P-phosphate. The plants were extracted with ethanol evaporated and paperchromatographied.

The experiments were carried out by seedlings kept in the dark or in the light for 24 hours. From the beginning of the experiments five samples were worked up.

The results showed, how the ^{32}P incorporated into the separated fraction during the incubation and what the correlation was between activity and the light effects.

Experimental Methods and Materials

The seven-ten days old seedlings of *Pisum sativum „Express"* were applied for the experiments. The plants were grown in sand, in greenhouse, at 22—24°C, on sunshine. Before the incubation process the plants were put into a K n o p solution diluted tenfold for two hours. During the experiments they were placed into a K n o p solution containing 15 μC/ml $KH_2{}^{32}PO_4$.

After incubation the reaction was stopped by adding to it hot ethanol and the plants were homogenised and extracted with 70 % ethanol and water for eight hours. The extract was chromatographied in two different solutions; I. n-butanol: n-propanol:acetone:formic acid 80 %:trichloro-acetic acid 30 % 8:4:5:5:3; II. n-butanol:n-propanol:acetone:ammonia 25 %:water 7:3:3:8:1.

The identification of spots was carried out on the basis of their R^f -value, and comparing them with standards, after spraying with different reagents: anilinphtalate, ammoniummolybdenate for sugarphosphates; $HgCl_2$-eozin solution and Wood-reagent for nucleotids; and by autoradiography.

The radioactivity of chromatograms was measured by scaler apparatus with GM-tube, the width of the slit was 0,5 cm. the activity of the following fractions has been decided: 1) inorganic phosphate, 2) ester-phosphate, 3) „indole"-phosphate, 4) nucleotide-phosphate.

The inorganic phosphate compound was ortho-phosphate. The compounds of nucleotid fraction were mainly ADP (adenosine diphosphate) and ATP (adenosine triphosphate). Sugar phosphates, glyceric acid phosphates, and AMP (adenosine monophosphate) belonged to the ester-phosphate fraction. The compounds of the „indole"-phosphate fraction, have given characteristic indole, sugar- and phosphate reactions. The compounds of the nucleotide fraction, have shown characteristic purine, sugar and phosphate reactions and the R^f -values of them were similar to the nucleotide phosphates. On the basis of the examination these compounds have been derivatives of indoleacetic acid.

Experimental Results

1. The Change of the Total ^{32}P activity in the Roots and in the Shoots of Plants According to the Time and Light Conditions

Eight-days old pea seedlings were kept in a K n o p solution diluted tenfold, containing 15 μC/l ^{32}P phosphate at 22 °C in darkness and the other series of pea seedlings were kept in the same condition, and they were illuminated with 4000 lux intensity for 24 hours. During this time the

samples were extracted after 2, 6, 12, 18, 24 hours with ethanol and chromatographied. Fig. 1 shows the total activity concerning one g fresh weight of plant material.

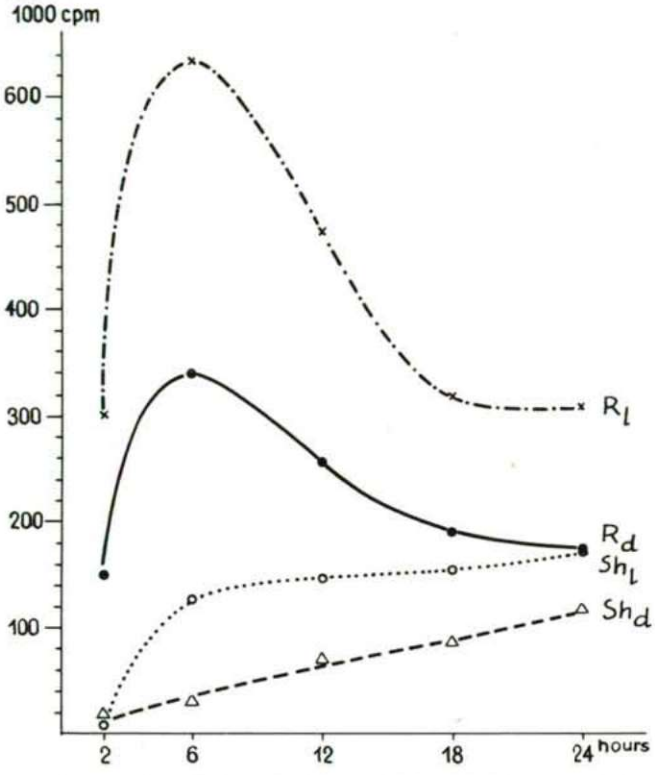

Figure 1. The total activity of the alcohol soluble ^{32}P incorporated into the roots and shoots of pea seedling according to the time and light conditions.
R_l = roots of illuminated seedlings
R_d = roots of seedlings kept in the dark
Sh_l = shoots of illuminated plants
Sh_d = shoots of seedlings kept in the dark

According to the result of experiments the incorporation of ^{32}P during the incubation period was always higher in the roots, than in the shoots. The roots and shoots of illuminated plants showed higher activity than the plants kept in the dark. The maximum of the total activity occurs after 6 hours.

During the time that has been studied the total activity of phosphate compounds of the shoots didn't give a maximum curve. The total activity increased at the beginning of experiments faster than it increased later. The raising of ^{32}P activity in the shoots kept in the dark was continuous. The incorporation of ^{32}P was almost linear. The curves of total activity have not shown significant changes between eighteen and twenty four hours.

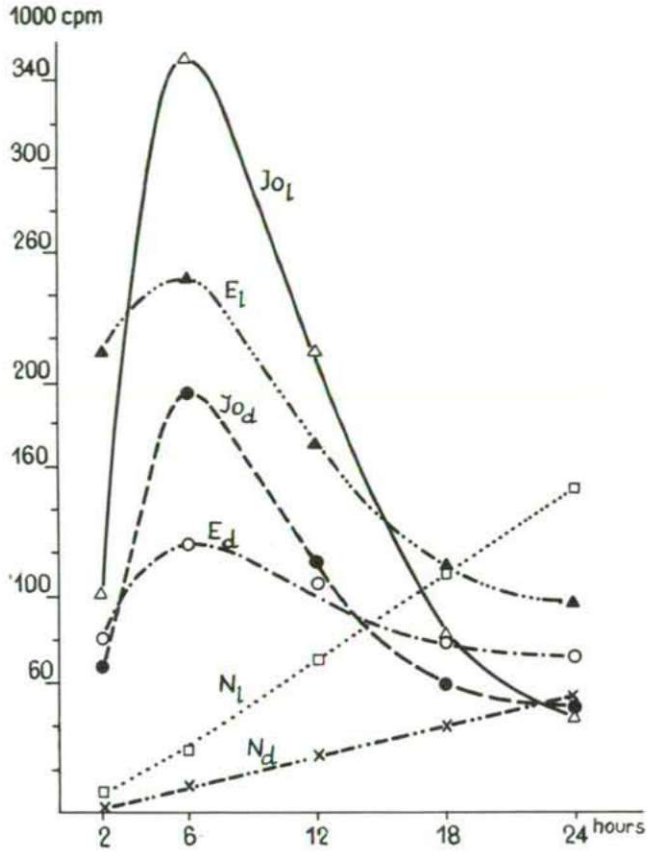

Figure 2. Radioactivity of the alcohol soluble ^{32}P fractions of pea shoots according to the time and light conditions.
Io_l = inorganic phosphate in the light
Io_d = inorganic phosphate in the dark
E_l = ester phosphate in the light
E_d = ester phosphate in the dark
N_l = nucleotide phosphate in the light
N_d = nucleotide phosphate in the dark

2. The Change of the ^{32}P Activity of Roots and Shoots during the Experiments in the Case of Different Light Conditions.

Fig. 2 shows that the amount of inorganic phosphates increased continuously whether the seedlings were kept in the dark or in the light. During the incubation period the activity of ester-fraction increased similarly, but the total activity was higher in the seedlings kept in the light, than it was in darkness. During the experiments the curve of phosphate compound didn't show any maximum.

Fig. 3 shows that the activity of the roots was higher than it was in the same fractions of the shoots. After six hours the amounts of the inorganic phosphates and of the organic phosphate ester fractions

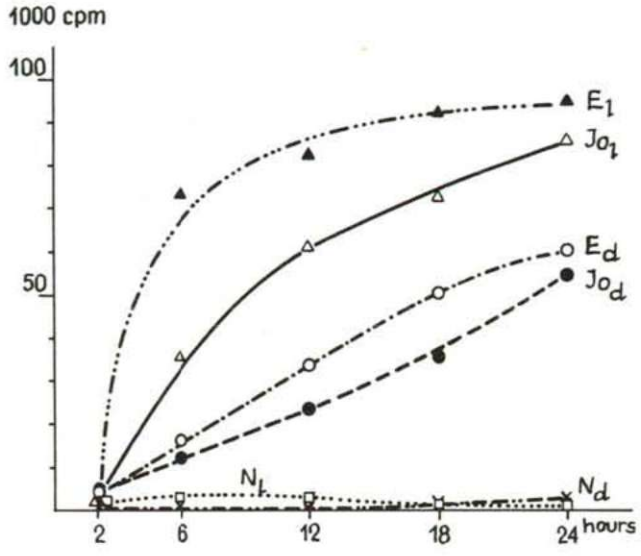

Figure 3. Radioactivity of the alcohol soluble ^{32}P fractions of pea roots according to the time and light.

Io_l = inorganic phosphate in the light
Io_d = inorganic phosphate in the dark
E_l = ester phosphate in the light
E_d = ester phosphate in the dark
N_l = nucleotide phosphate in the light
N_d = nucleotide phosphate in the dark

of the roots show a maximum on the basis of activity. The maximum was higher in the roots of seedlings kept in the light, than it was in the other case. After the maximum the activities of both fractions decreased and an equilibrium occurred after 18—24 hours.

The phosphate incorporation into the nucleotid fraction was continuous in both cases in the roots and similarly in the shoots during the experiments.

The activity of „indole"-phosphate fraction showed unambiguous increasing tendency when the seedlings were kept in the dark.

3. The Ratio of Single Fractions in the Percentage of Total Activity According to the Time and Different Light Conditions.

For the explanation of the experiments it was very important to compare the ratios of the measured activity of single fractions in the percentage of alcohol soluble ^{32}P activity. The data calculated in this way are in Table 1 and Fig. 4.

According to the data the calculated ratio of activities of the shoots of seedlings kept in the dark had a maximum of inorganic phasphates before the first sample was measured. This value was constant for 6—18 hours and it increased slightly after this period.

TABLE 1. Incorporation of ^{32}P into the different phosphate fractions of the shoots and the roots of pea seedlings according to the time and light conditions

Fraction		2h		6h		12h		18h		24h	
		C_{pm}/g	%	C_{pm}/g	%	C_{pm}/g	%	C_{pm}/g	%	C_{pm}/g	%
S_d	Io	5642	54,53	12178	41,54	23506	40,78	35679	40,42	54296	46,00
	E	4585	44,31	16987	57,95	33040	57,32	50416	57,12	60842	51,55
	I	88	0,85	86	0,29	216	0,37	322	0,36	457	0,38
	N	31	0,30	65	0,22	880	1,53	1844	2,09	2446	2,07
	T	10346		29316		57642		88261		118041	
S_l	Io	2087	40,79	44996	35,62	61410	42,10	73240	46,66	88482	50,47
	E	1909	37,31	76372	60,45	78804	54,03	82571	52,60	85736	48,91
	I	76	1,49	2831	2,24	3200	2,19	980	0,62	632	0,36
	N	1044	20,41	2140	1,69	2445	1,68	1218	0,78	452	0,25
	T	5116		126339		154859		157000		175302	
R_d	Io	67979	44,00	195249	57,22	116210	45,91	59407	32,25	46793	26,22
	E	81569	52,80	124217	36,40	104060	41,12	79202	42,90	72226	40,46
	I	1061	0,69	4501	1,32	4805	1,90	5112	2,78	5960	3,34
	N	3885	2,51	17262	5,06	28004	11,06	40473	21,97	53498	29,58
	T	154494		341229		253079		184194		178477	
R_l	Io	100117	30,88	349763	54,69	214520	54,87	81420	25,42	42335	13,74
	E	215525	66,48	248864	38,91	170218	36,40	114042	35,59	97575	31,67
	I	935	0,29	10949	1,71	12602	2,69	14806	4,62	18457	5,99
	N	7608	23,45	29982	4,69	70320	15,04	110129	34,37	148752	48,60
	T	324185		639558		476660		320397		308109	

S_d = shoots of seedlings kept in the dark
S_l = shoots of illuminated seedlings
R_l = roots of illuminated seedlings
R_d = roots of seedlings kept in the dark

Io = inorganic phosphate
E = ester phosphate
N = nucleotide phosphate
I = „indole"-phoshate

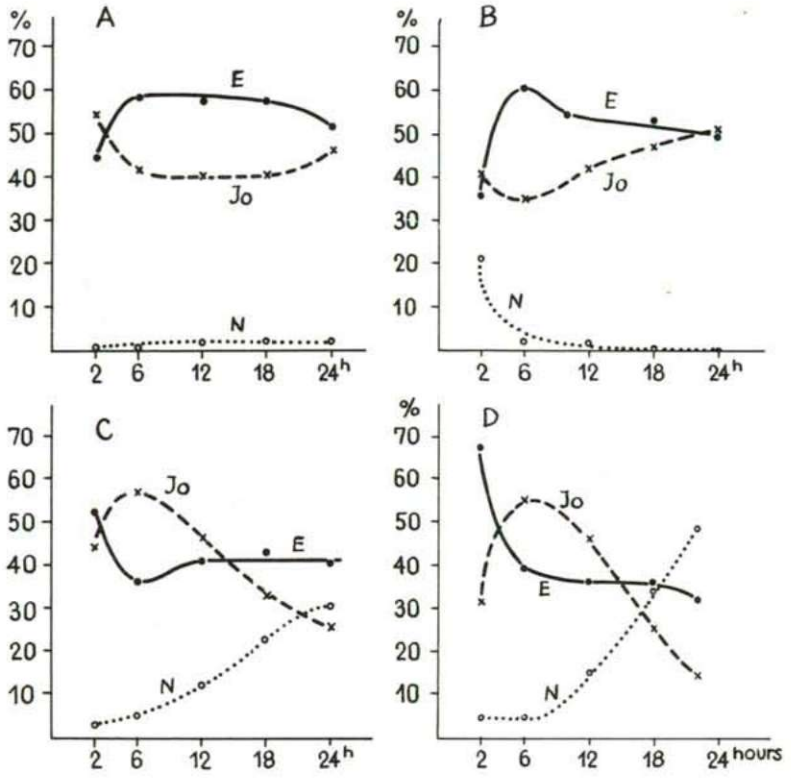

Figure 4. Radioactivity of phosphate fractions in the percentage of the incorporated total ^{32}P according to the time and light conditions.

Io_l = inorganic phosphate in the light
Io_d = inorganic phosphate in the dark
E_l = ester phosphate in the light
E_d = ester phosphate in the dark
N_l = nucleotide phosphate in the light
N_d = nucleotide phosphate in the dark

The curve of ester fraction didn't show any characteristic maximum but the percentage of the incorporated ^{32}P was almost continuous. The participation of the nucleotide fraction in the total activity raised slowly and fluently. After a time the activity of them was about constant. The illuminated seedlings showed similar change but it was significant that the activity of nucleotide fraction had a maximum before the examined first sample (Fig. 4B). The percentages of ^{32}P fractions of the roots kept in the dark or in the light were approximately similar. The changes of the inorganic and of the nucleotide fractions were strong and of contrary character (Fig. 4C and D).

Discussion

A programme of the present work was to select the suitable parameters for the examination of some physiological processes. We have found a method that gave possibility to separate the phosphate compounds and to establish the qualitative and quantitative ratios of them at different conditions.

Similar experiments were carried out by Simonis and Weichart in 1958, Weichart in 1961 with Helodea, by Loughman in 1960 with potato parenchyma tissues and at least by Heitefuss in 1961 with the leaves of wheat and with intact wheat plants.

The mentioned papers described experiments and results after a short few minutes incubation period or after a few days incubation. The authors have not examined yet how the phosphate concentration changed in a few hours period. For the registration of experimentally inducated growth the above mentioned period was selected because the 10—20 minutes experiments didn't give valuable data about the growth. After a longer period there were a lot of side reactions, which could disturbe the results.

On the basis of the experiments the authors established that the total activity of ^{32}P fractions and the percentile participation of phosphate compounds in the case of examined seedlings showed more or less change either in the light or in the dark in the first 6—8 hours period. After 6—8 hours an equilibrium formed, that didn't change for 18—24 hours. This result was very significant for the seedlings kept in the dark.

There was a maximum of ^{32}P activity in the roots after six hours. The activity decreased quickly after this period, because the fast uptake of inorganic phosphate was followed by transportation into the shoots and by formation of alcohol insoluble compounds.

In the shoots the total activity increased continuously, however the percentile amount of inorganic and esterphosphates were approximately constant after a few hours. The effect of the light increased the total activity of phosphate compounds and it also increased the formation of free nucleotides. Higher acitivity of nucleotides was found also in the roots.

The experiments have given a view about the dynamics of the incorporation of ^{32}P and how the light effect changed the phosphate incorporation. The experiments have given a lot of data about the transport of phosphate compounds that was not explained in detail.

On the basis of experiments the authors established that for studying the effects of growth regulators the seedlings kept in the dark are suitable.

Summary

It was examined how the qualitative and quantitative ratios of phosphate compounds changed with the light effects in a 24 hours period. Results of the experiments showed that the light effects changed on differential way the total acitivity of the roots and the ratio of differential phosphate compounds. There was always found a higher acitivity in the roots than in the shoots. The time curve of activities measured in the root extract gave a maximum, while the incorporation into the shoots was continuous. The light increased the incorporation of ^{32}P both into the roots and into the shoots. In the roots the Cpm activity of the inorganic phosphates decreased after six hours calculated by the separated fractions of phosphate compounds. Similarly the percentile activity of inorganic phosphate decreased after six hours. The activities of organic fraction increased fluently. Probably they formed from the incorporated inorganic phosphates. This transformation resulted a decreasing of the activity of inorganic phosphates.

These experiments gave at first data for the qualitative and quantitative change of so-called „indole"-phosphate fraction. These compounds can be very important physiological active compounds. Into this fraction the incorporation of ^{32}P increased continuously in the roots with the time. During the experiments the „indole"-phosphate fraction of the shoots didn't show any unambiguous tendency. On the basis of the mentioned character of „indole"-phosphates, they are similar to the nucleotide fraction.

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