ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. 53, nr 3: 385-398
1984

The effect of sodium humate on the content of different types of phosphorus compounds and on the activity of acid phosphatases in tomato seedlings

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(Received: December 8, 1983. Accepted: February 14, 1984)

Abstract

The effect of sodium humate on the level of some fractions of phosphorus compounds in the leaves and roots of tomatoes cultured in mediums with a full or reduced phosphorus dose was studied. A dependence was found between the presence of humate in the medium and an increase in the amount of some organic phosphorus compounds in the leaves (lipid phosphorus, easily hydrolyzed phosphorus compounds, nucleic acid phosphorus). In addition, humate lowered the activity of acid phosphatases which had been excessively increased due to the plants inadequate phosphate supply. The results suggest that Na-humate affects phosphorus metabolism not only in an indirect manner, but also directly through penetration of humic substances into the plant tissues.

Key words: sodium humate, phosphorus compounds, acid phosphatases

INTRODUCTION

Studies conducted by several authors (Ȟajdukovič and Ulrich 1965, Jelenič et al. 1966, Gumiński et al. 1983) working on the effect of humic substances on the phosphorus nutrition of plants, concentrated mainly on showing the increased uptake or accumulation of phosphorus by different species of plants in the presence of humate, especially under conditions of phosphorus deficiency or its recession in the roots environment. In previous papers (Lisiak 1978, 1984) the effect of certain environmetal factors such as Ca^{2+} and Fe^{3+} concentration and the pH of the medium on the effectiveness of sodium humate on the plants phosphate nutrition was studied. It was found that humate increased both the accumulation of phosphorus

in tomato shoots and the dry mass yield, as well as, eased to a large degree the symptoms of deficiency of this macroelement in the plant.

Flanderkova et al. (1969) made a certain attempt to determine more precisely the changes which take place in the phosphorus metabolism of *Scenedesmus quadricauda* as a result of the action of humic substances. The studied presented in this paper on the activity of acid phosphatases and changes in the level of phosphorus compounds were carried out in order to gain information on the type of disturbances in the phosphorus metabolism occuring in plants given insufficient amounts of phosphorus and on the role of sodium humate in the phosphorus nutrition of higher plants.

MATERIAL AND METHODS

The experiments were conducted on the "Mory 33" variety of tomatoes grown in water cultures in an unacclimatized green-house during the summer months of June and July. The plants were cultured as described in a paper by Lisiak (1978). The mediums composition in $g \cdot dm^{-3}$ of distilled water was as follows: 0.71 $Ca(NO_3)_2 \cdot 4H_2O$, 0.57 KNO_3, 0.284 $MgSO_4 \cdot 7H_2O$, 0.142 $(NH_4)_2HPO_4$, 0.116 $Fe_2SO_4 \cdot 9\,H_2O$ with microelements in $mg \cdot dm^{-3}$ of medium: H_3BO_3 — 1.54, $ZnSO_4 \cdot 7H_2O$ — 0.57, $CuSO_4 \cdot 5H_2O$ — 0.11, Na_2MoO_4 — 0.12, $MnCl_2 \cdot 4H_2O$ — 0.97. After ten days of culture, some of the plants were transferred to the medium with the full phosphorus dose (1 P) and part, to the medium with the amount of this macroelement lowered 10 times (0.1 P), with or without the addition of humate. In the medium with the lowered phosphorus content, the ammonium nitrogen level was maintained by adding the appropriate amount of NH_4Cl.

Sodium humate was obtained from one-year-old leaf compost as desribed by Gumiński (1950) and dissolved in 0.01 N NaOH. Ten cm^3 of this solution containing 100 mg of humate, were added to 1 dm^3 of medium. The pH of the medium was set at 6.4 using 0.1 N HCl or 0.1 N NaOH. The mediums were mixed and aerated daily by pouring them into previously prepared jars.

After 3, 6 and 9 days cultivating the plants in mediums which differed from each other in respect to their phosphorus content and the presence or absence of humate, the level of individual phosphorus compound fractions and the activity of acid phosphatases were assayed in the leaves and roots of the test plants.

The different types of phosphorus compounds were determined in fresh plant material (Mejbaum-Katzenellenbogen and Mochnacka 1966).

One gram of leaves or roots (previously washed in distilled water) were ground in a mortar with 2 cm^3 10% trichloroacetic acid (TCA). Next, the homogenate was transferred quantitatively to a cylinder and brought to a constant volume using 5% TCA, so that the final acid concentration in the sample was 6%. The extract was left standing for 15 minutes during which it was mixed often. Next it was filtered through gauze and centrifuged. All of the steps were carried out in 0 to +4°C. The phosphorus which was soluble in acid in the cold and was found in the supernatant was assayed by the method of Fiske and Subbarow (1925):

— inorganic phosphorus (P_i) — directly in the extract,
— easily hydrolyzed phosphorus compound (P_{eh}) — after previous hydrolysis for 7 min in a boiling water bath,
— dificultly hydrolyzed phosphorus compounds (P_{dh} — after digestion of the sample in 2 N H_2SO_4 and 2 N HNO_3.

Lipid phosphorus (P_{lip}) was assayed in the sediment left after extraction with TCA. Five cm^3 ethanol: diethyl ether (3:1) were added to the pellet and placed in a boiling water bath for 30 sec. The procedure was repeated, both extracts were combined, filtered and brought to a constant volume. Next, the solvent was evaporated from a measured part of the extract and the resulting solid was digested in 2 N H_2SO_4 and 2 N HNO_3 after which the phosphorus content was determined by the method of Fiske and Subbarow (1925).

The total phosphorus content (P_t) was determined by digesting in concentrated H_2SO_4 and HNO_3 the same mass of fresh material as was used for extraction of acid (TCA)-soluble phosphorus compounds. Organic phosphorus (P_o) was calculated as the difference between P_t and P_i. The fraction of phosphorus which was built into nucleic acids was calculated as the difference between $(P_o\text{-}P_{dh})\text{-}P_{lip}$.

Acid phosphatases were extracted using 0.01 M acetate buffer, pH 5.0, containing 0.25 M sucrose. Leaves or roots (washed in distilled water) were homogenized in a porcelain mortar using 5 cm^3 buffer per 1 g material. The extract was filtered through two layers of gauze and centrifuged for 10 min at 1500 × g. The pellet (fraction I containing cell wall elements) was suspended in 5 cm^3 of extraction buffer. The liquid from above the pellet was again centrifuged, this time, at 20 000 × g for 15 min. The supernatant from the second centrifugation was the soluble fraction (II). The steps described above were carried out at 0 to +4°C. The volume of the incubation mixture was 3 cm^3, the substrate used was 3 mM p-nitrophenyl phosphate (pNPP). To 2.4 cm^3 of acetate buffer, pH 5, 0.4 cm^3 of the enzyme-containing extract and 0.2 cm^3 substrate were added. This was incubated for 30 min at 37°C. After this time the reaction was stopped by adding 1 cm^3 of cold TCA and leaving the samples in the cold for 15 min. Next, the precipitate was centrifuged off and the freed inorganic

phosphate was determined in the supernatant by the Fiske and Subbarow method (1925). The enzyme activities were expressed in nmoles of inorganic phosphorus freed from p-nitrophenyl phosphate per gram of fresh mass per minute.

RESULTS

The content of phosphorus-compound fractions in the organs of the test plants changed significantly along with the lowering of the phosphorus dose, and was modified by the presence of humate in the medium. The total phosphorus content in the leaves and roots of the plants was as presented on Fig. 1. In the plants from the 0.1 P combination picked on the third

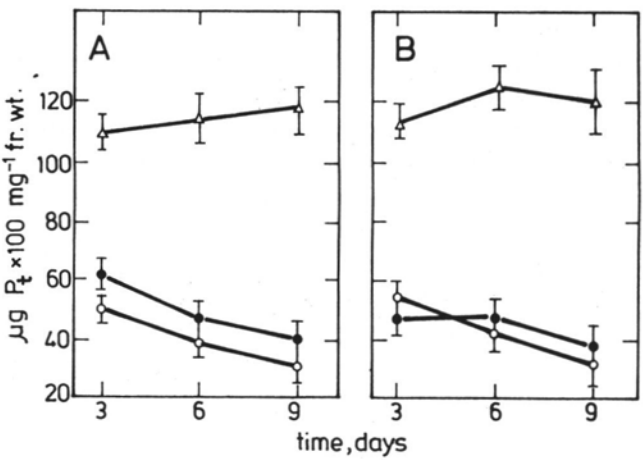

Fig. 1. Changes in the total phosphorus (P_t) content in the fresh mass of leaves (A) and roots (B) of tomatoes grown on the full and reduced dose of phosphorus in the medium with Na-humate added. The vertical bars denote ± SE of average values from 3 repetitions. –Δ–control (1 P), –o–0.1 P dose, –●–0.1 P dose + humate

day of cultivation, a significant lowering of the phosphorus content compared with the control took place, and this drop continued during the next days of the experiment. The total phosphorus content in the leaves of plants from the 0.1 P combination analyzed on the third day, rose as an effect of humate. In the roots, initially (on the 3rd day) the phosphorus content in the combination with humate was lower than without it, and only on the 6th and 9th days it rise as an effect of humate. These differences are, however, within the limit of error.

The level of inorganic phosphorus (P_i) was in the studied plant organs in the 0.1 P combination much lower than in control plants (Fig. 2). In

combination with a low phosphorus content in the medium, humate increased the amount of inorganic phosphorus in the leaves and lowered it in the roots; the differences are significant on the 3rd day of cultivation.

The leaves and roots of plants in the 0.1 P combination contained considerably less organic phosphorus (P_o — Fig. 3) and a slight declining

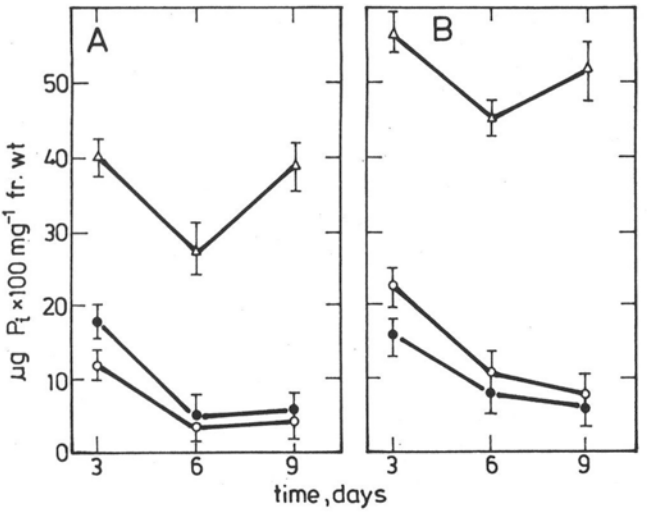

Fig. 2. Changes in the inorganic phosphorus (P_i) content in the fresh mass of leaves (A) and roots (B) of tomatoes grown on the full and reduced phosphorus dose in the medium with Na-humate added. Symbols as in Fig. 1

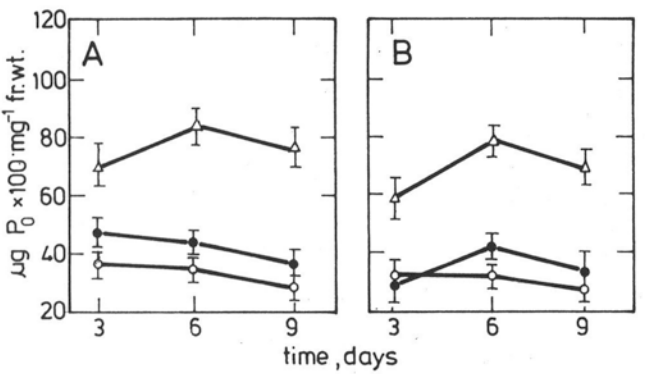

Fig. 3. Changes in the organic phosphorus (P_o) content in the fresh mass of leaves (A) and roots (B) to tomatoes grown on the full and reduced phosphorus dose in the medium with Na-humate added. Symbols as in Fig. 1

tendency was maintained in time (6th and 9th days). Humate caused an increase in the organic phosphorus content in the leaves of seedlings in the 0.1 P combination starting on the 3rd day, and slightly later in the roots — from between the 3rd and 6th days.

Table 1

The ratio of organic to inorganic phosphorus (P_o:P_i) in the tomato leaves and roots

Treatment	Leaves	Roots
	after 3 days	
0.1 P	3.2	1.2
0.1 P+humate	2.9	1.8
1 P	1.7	1.0
	after 6 days	
0.1 P	12.1	2.8
0.1 P+humate	9.9	5.1
1 P	3.0	1.6
	after 9 days	
0.1 P	7.7	3.4
0.1 P+humate	8.8	4.9
1 P	1.8	1.2

The ratio of organic to inorganic phosphorus (P_o:P_i — Table 1) was calculated on the basis of the data from the above experiments. The numerical values of this ratio in the combination with the full phosphorus

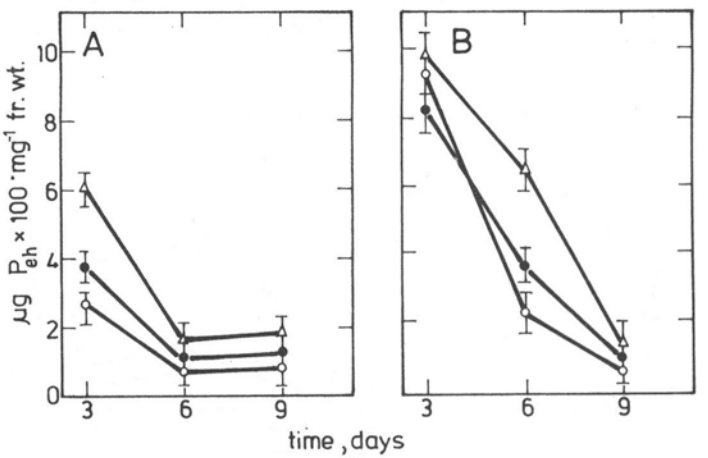

Fig. 4. Changes in the content of easily hydrolyzed phosphorus (P_{eh}) compounds in the fresh mass of leaves (A) and roots (B) of tomatoes grown on the full and reduced phosphorus dose in the medium with Na-humate added. Symbols as in Fig. 1

dose in the medium (1 P) are higher in the leaves than in the roots and rise between the 3rd and 6th days of the experiment. However, in the leaves of plants with symptoms of phosphorus deficiency, this ratio rises sharply to the 6th day, after which it falls between the 6th and 9th days; in the

roots the tendency to rise is maintained throughout the experiment. Humate caused an increase in the P_o:P_i ratio in the roots of plants from the 0.1 P combination and lowered it in the leaves analyzed on the 3rd and 6th days of the experiment.

The fraction of easily hydrolyzed phosphorus compounds (P_{eh}) contained in the leaves of seedlings given a low dose of phosphorus was already significantly lower by the 3rd day of cultivation and remained on a low level in the 0.1 P and control combinations on the 6th and 9th days (Fig. 4). A significant increase in the P_{eh} content in the leaves of seedlings from the

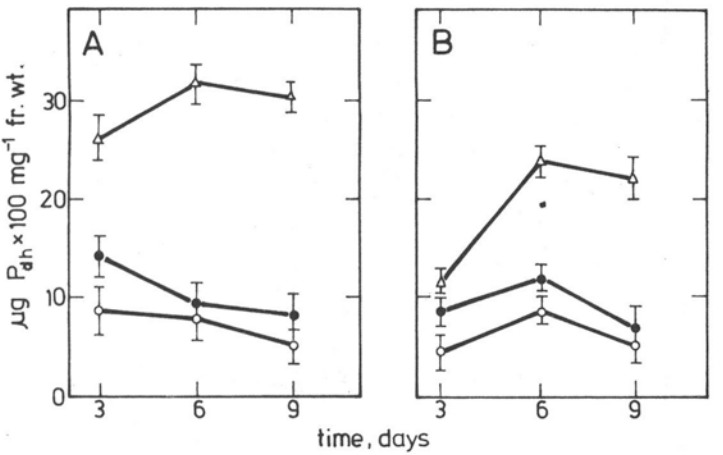

Fig. 5. Changes in the content of difficultly hydrolyzed phosphorus (P_{dh}) compounds in the fresh mass of leaves (A) and roots (B) of tomatoes grown on the full and reduced phosphorus dose in the medium with Na-humate added. Symbols as in Fig. 1

0.1 P combination took place in response to sodium humate on the 3rd day of the experiment. In the roots, changes in this phosphorus fraction followed a different pattern. In these organs, a sharp decline in the amount of P_{eh} took place in all combinations between the 3rd and 9th days. After 3 days, the reduced dose of phosphorus caused a slight lowering of the content of this fraction of phosphorus compounds and the addition of humate lowered its level even more. After 6 days, humate increased the P_{eh}content in the roots of plants with a phosphorus deficiency.

The level od difficultly hydrolyzed phosphorus compound (P_{dh} — Fig. 5) decreased significantly most notably in the leaves but also in the roots of plants which were grown for 3 days in the medium with the reduced amount of phosphorus; this decreased level of P_{dh} was maintained on the 6th and 9th days of cultivation. The presence of sodium humate in the medium caused, under these conditions, an increase in the P_{dh} fraction

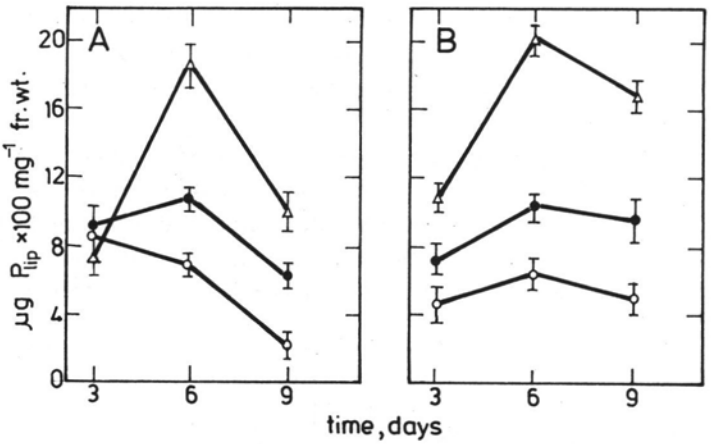

Fig. 6. Changes in the lipid phosphorus (P_{lip}) content in the fresh mass of leaves (A) and roots (B) of tomatoes grown on the full and reduced phosphorus dose in the medium with Na-humate added. Symbols as in Fig. 1

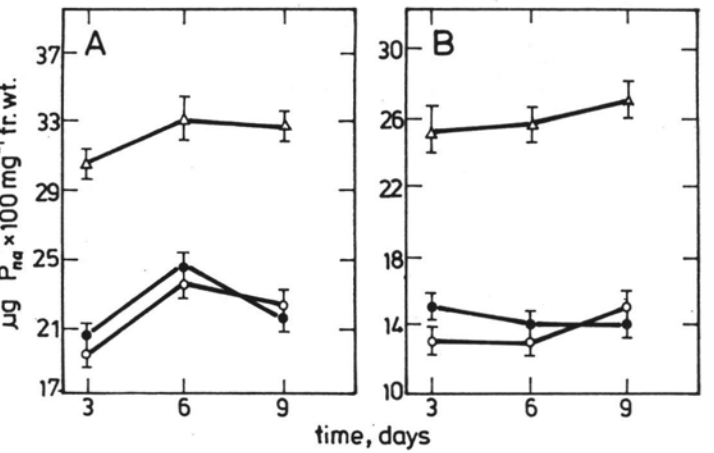

Fig. 7. Changes in the nucleic acid phosphorus (P_{na}) content in the fresh mass of leaves (A) and roots (B) of tomatoes grown on the full and reduced phosphorus dose in the medium with Na-humate added. Symbols as in Fig. 1

both in the leaves, as well as, in the roots of the plants from the studied combination, especially on the 3rd and 6th days of cultures.

Significant differences in the lipid phosphorus content (P_{lip} — Fig. 6) between combinations 1 P and 0.1 P were seen in the case of the underground organs on the 3rd day, in the leaves only on the 6th and 9th days of culture. When sodium humate was included in the medium with the reduced phosphorus content, the level of lipid phosphorus in the roots greatly increased starting from the 3rd day, in the leaves only from the 6th and 9th days.

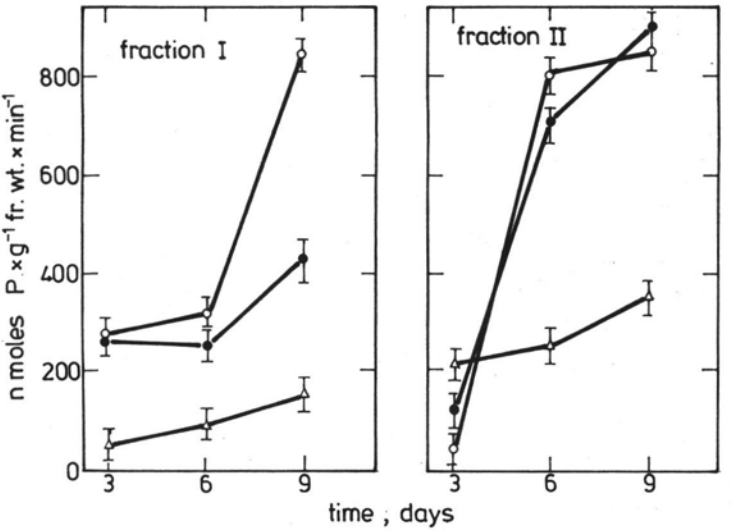

Fig. 8. The total activity of acid phosphatases in the leaves of tomatoes grown in mediums with the full or reduced phosphorus dose and Na-humate added. Symbols as in Fig. 1

The amount of phosphorus built into nucleic acids (P_{na} — Fig. 7) was already significantly lower in plants given an insufficient phosphate supply (0.1 P) on the 3rd day of growth. In this combination, in response to humate, a slightly elevated level of phosphorus in this fraction was maintained in the leaves and roots between the 3rd and 6th days of the experiment.

The total activity of the acid phosphatases in the leaf extracts from tomato plants insufficiently supplied with phosphates was already elevated in fraction I on the 3rd day of the experiment and rose sharply between the 6th and 9th days (Fig. 8). The presence of humate in the medium with the low phosphorus dose caused a significant lowering of the phosphatase activity in fraction I especially after 9 days of culture. In the soluble fraction (II) the total enzyme activity in the leaf extracts was in the 0.1 P combination on a level lower than the control on the 3rd day and rose sharply until the 6th day, remaining on a high level on the 9th day also. In seedlings growing on the 0.1 P + humate medium, the total acid phosphatase activity in the soluble fraction was only slightly lowered on the 6th day with a small rise at the begining. In the roots, the activity of enzymes in fraction I followed a similar pattern as in the leaves (Fig. 9). On the 3rd day of cultivation in the 0.1 P combination, it was higher than in the control where it was on a low level during the entire study. On the 6th and 9th days cultivation the activity of acid phosphatases in the 0.1 P combination rose significantly. At this time, the effect of humate, which depended on lowering the enzyme activity, became visible. In the soluble fraction on the other hand, the greatest differences in the phosphatase

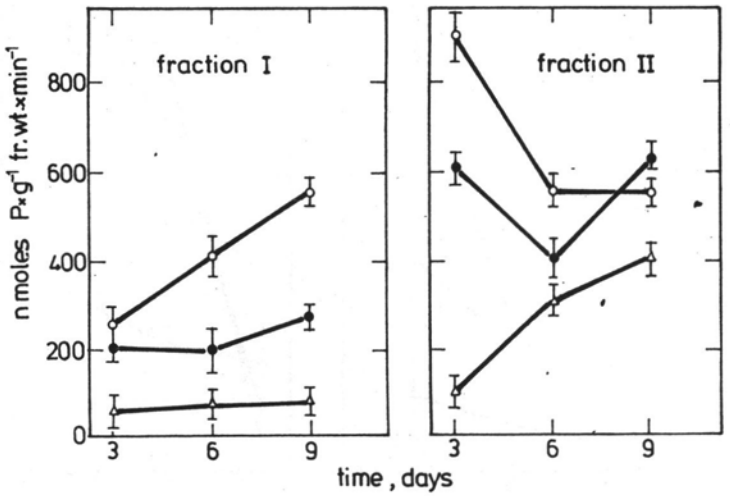

Fig. 9. The total activity of acid phosphatases in the roots of tomatoes grown in mediums with the full or reduced phosphorus dose and Na-humate added. Symbols as in Fig. 1

Tabela 2

The yield of dry mass (in g) from three tomato seedlings from one jar during 9 days of culture in the medium with the full (1 P) and lowered (0.1 P) amount of phosphorus

Treatment	Shoots	Roots
	after 3 days	
0.1 P	0.118 a	0.026 a
0.1 P+humate	0.134 a	0.034 b
1 P	0.165 a	0.033 b
	after 6 days	
0.1 P	0.231 b	0.058 c
0.1 P+humate	0.257 b	0.059 c
1 P	0.313 b	0.063 c
	after 9 days	
0.1 P	0.307 b	0.075 c
0.1 P+humate	0.506 c	0.121 d
1 P	0.740 d	0.134 d

Average values from 6 repetitions. The differences between the averages denoted by different latters are proven statistically with a 95% probability.

activities between the control and studied combinations were found on the 3rd day of culture. After 6 days, a significant lowering of the activity in combination 0.1 P occured, and remained on the same level to the 9th

day. The presence of sodium humate in the medium with a phosphorus deficit caused a significant lowering of the activity of acid phosphatases in fraction II only during the 3rd and 6th days of the experiment.

The results of chemical and enzymatic analyses of plant material presented above were supplemented by the measurements of dry mass yield of seedling shoots and roots (Table 2). After 3 days of growth on mediums differing in respect to their phosphorus content, slight differences in the dry mass of shoots and roots began to become apparent. After 6 days, significant difference in the yield of seedling root dry mass still had not made themselves evident; in the case of shoots, a small drop in the yield in the 0.1 P combination compared with the control took place. The greatest differences were seen on the 9th day when the dry mass yield of shoots and roots in the 0.1 P combination was greatly reduced in comparison with the control. Between the 6th and 9th days of the study, a clear-cut effect of sodium humate stimulation of the dry mass yield of the shoots and roots of plants insufficiently supplied with phosphorus was found.

DISCUSSION

The results of short-term experiments indicate that the changes in the dry mass yield of plants, seen on the 9th day of cultivation in a medium with a low phosphorus dose are preceded by at least 6 days of disturbances in the metabolism of phosphorus compounds. Therefore, the reasons for the better growth of plants insufficiently supplied with phosphorus in the presence of humate should be sought in the initial phase of cultivation.

The decrease in the inorganic phosphorus content which took place in the test plants is considered one of the first signs of phosphorus deficiency (Roux 1966). If humate increased the pool of inorganic phosphorus, its effect could be explained by an indirect effect on plants through improvement of the conditions for phosphorus uptake in the external environment. Instead, the inorganic phosphorus level is higher only in the leaves, whereas in the roots, which are in direct contact with the medium containing humate, the inorganic phosphorus level is lowered. Most likely, phosphates are transported from the roots to the assimilating organs. This is also pointed to by the results of Tichy (1980). This does, to some extent, act to lower the sharply rising organic to inorganic phosphorus ratio in the leaves of seedlings displaying a phosphorus deficit (Table 1).

The inorganic phosphorus which is transported to the leaves is incorporated into organic compounds, whose content in the leaves rises sooner than in the roots (Fig. 3). Form among the organic phosphorus compounds in the leaves of seedlings with a phosphorus deficit, after the first three days of cultivation in the presence of humate, an elevated level of easily

hydrolyzed phosphorus compounds (ATP, ADP, glucose-6-phosphate — Fig. 4) is maintained. The same is found for difficultly hydrolyzed phosphorus compounds — coenzymes, mononucleotides, triose phosphates, phosphopyruvic acid, phosphoglyceric acid, carbohydrate phosphate esters (Fig. 5). The raised level of those phosphorus compound fractions is correlated with the relatively low activity of phosphatases (Fig. 8) in the initial days of cultivation. In the roots of plants insufficiently supplied with phosphorus, humate raised the low level of the difficultly hydrolyzed fraction and later counteracted the abrupt fall in the easily hydrolyzed phosphorus compound fraction.

The consequence of insufficient phosphorus supply to plants is, among others, the lowering the amount of phospholipids. This was found in studies by Bieleski (1972) on *Spirodela* and Roux (1966) on tomatoes, and has also been confirmed by the experiments conducted in this study (Fig. 6). The optimum level of phospholipids, which are a component of cell membranes, determines the proper functioning of the latter in the uptake of minerals, in the processes of biological oxidation localized in the mitochondria and in photosynthesis which take place in chloroplasts. The significantly elevated level of phospholipids in the leaves and roots of plants as an effect of sodium humate surely improved the conditions in which the basic physiological processes take place. In the studies conducted here, a positive effect of humate on the phosphorus content of nucleic acids in the leaves and roots of the studied seedlings was found during the early days of culture. Similar effects were found by Fialova (1969) in the roots of wheat and Christeva et al. (1971) in beans and sunflowers. The increasing of the nucleic acid content by humate is explained by Christeva as direct intervention of humate in the plant metabolism. According to her, after entering the cells, humate increases the intensity of oxidative phosphorylation. This phenomenon is also confirmed by the studies of Milczarek (1983). In the literature on the physiological effects of humic substances there is little data on their effect on the metabolism of specific phosphorus compounds. Flanderkova et al. (1969) described the positive effect of fulvic acids on the content of easily and difficultly hydrolyzed phosphorus and on total phosphorus in *Scenedesmus*. In respect to the fulvic acids which are characterized, among others, by a smaller molecules, there are more experimental data indicating the possibility of these compounds entering into the plant cell. Sodium humate, used in these experimets in a rather high concentration, surely had an indirect effect on plants due to its colloidal and complexing properties. It does not seem necessary for humic substances to enter the cell in order to cause changes in the phosphorus metabolism. Probably only contact of humate with the enzymes in the cell wall and plasmalemma is sufficient (Gumiński and Sulej 1979, Tretyn and Gumiński 1983). In the studies presented here the activity of acid phosphatases was lowered by the increase in the concentration of inorganic phosphates in the tissues

of the test plants, but could also have been inhibited directly by the presence of humate in the enzymatic reaction medium, as has been shown *in vitro* by Malcolm and Vaughan (1979). This fact could be used to explain the lowering of the activity of the acid phosphatases in the fraction of cell walls of tomato roots which were in the medium containing humate. This explanation of the drop in the activity in the soluble fraction of root cells and in both seedling leaf fractions in response to humate would necessitate the acceptance of the assumption that humate enters into the cells and is transported to the above-ground parts of plants.

Acknowledgments

I would like to thank Prof. Stefan Gumiński for the proposition to undertake this topic and for his critical comments during the writing of this manuscript.
These studies were financed by IUNG in Puławy, problem No. 09.4.

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Wpływ humianu sodu na zawartość różnych związków fosforowych i aktywność kwaśnych fosfataz siewek pomidorów

Streszczenie

Badano wpływ niedostatecznego zaopatrzenia roślin w fosforany i obecności Na-humianu na poziom poszczególnych frakcji związków fosforowych i aktywność kwaśnych fosfataz. Stwierdzono zależność pomiędzy obecnością humianu w pożywce a podwyższeniem w liściach zawartości niektórych organicznych związków fosforowych (fosfor lipidowy, fosfor kwasów nukleinowych, związki fosforowe trudno hydrolizujące). Ponadto Na-humian obniżał aktywność kwaśnych fosfataz nadmiernie podwyższoną na skutek niedostatecznego zaopatrzenia roślin w fosforany. Wyniki sugerują, że oddziaływanie Na-humainu na gospodarkę fosforową roślin może odbywać się zarówno w sposób pośredni jak i bezpośredni po ewentualnym wniknięciu substancji próchnicznych do tkanek rośliny.