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Phosphorus Uptake Suppression in Maize Seedlings as Related to Humate Supply

V. TICHÝ

Department of Plant Biology, Faculty of Science, J. E. Purkyně University, Brno*

Abstract. The effect of sodium humate on phosphorus uptake by maize seedlings devoid of the endosperm was studied. Short-term experiments have shown that a suppression of phosphate ion absorption occurs under the described conditions in the presence of humate without decreasing the weight of experimental plants. The effect reported is delayed and is enhanced by prolonging the preliminary phase of starvation. It varies with the pH of the medium and reduces under extreme conditions the phosphate release from the root to the medium.

The action of humus substances favouring the growth of plants is very often explained as a stimulation of the uptake of mineral nutrients whose increased amount in the plant organism activates the other physiological processes (*cf.* for instance GUMIŃSKI 1967). According to FLAIG (1975) this kind of action of humus substances is most pronounced at extreme values of the factors of the outer environment; more generally, the manifestation of their activity depends on the influences of the medium to which the plant is being exposed.

We were able to corroborate this statement as regards the light effect (TICHÝ 1981a, TICHÝ and SALAJKOVÁ 1982) and the concentration of mineral nutrients in the substrate (TICHÝ 1980a, b, 1981b). We were, however, unable to confirm the above assertion in its general formulation, finding the response of plants to the presence of humate much more complex, weakened just under the extreme conditions to the point of extinction. As a result of our experiments we present this study dealing with the action of sodium humate on phosphorus uptake by maize seedlings after the removal of the endosperm and with a lowered pH.

MATERIAL AND METHODS

The experiments were performed with maize (*Zea mays* L.) CE 170 (father of the hybrid CE 200). The caryopses of this plant were soaked in tap water for 24 h, then transferred onto polyethylene pellets 3 mm in diameter, covered

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**Address:* Kotlářská 2, 611 37 Brno, Czechoslovakia.

with water leaving 1 cm below the level of the layer surface, and allowed to germinate for 3 days. Afterwards they were planted on stretched gauze with the roots submerged in tap water, where they grew for another 3 days. The illumination was provided by three 80 W fluorescent lamps from a distance of 35 cm. On the seventh day of the experiment the plants were deprived of the endosperm and subjected to starvation, the duration of which together with the medium in which it took place are given in the section Results and Discussion. The solution used in this phase of the 3rd experiment was prepared by a tenfold dilution of the stock solution of the following composition: In one litre of distilled water 2.5 mM $CsSO_4.2H_2O$ and 1 mM $MgSO_4.7H_2O$ are dissolved and 0.1 ml of solution by Hoagland is added. After the starvation phase elapsed, the experimental plants were placed in the KH_2PO_4 solution whose concentration was determined analytically during the experiment using the technique of FISKE and SUBBAROW (1925) modified by JIRÁČEK *et al.* (1967). The exact concentration level of potassium phosphate and sodium humate used in the individual experimental variants are also given in the following section. Sodium humate (henceforth NaHU) was obtained from peat by the procedure described in more detail in another volume of the present journal (TICHÝ 1981a).

RESULTS AND DISCUSSION

To avoid some complications associated with determining the phosphorus absorption rate and to increase the phosphorus concentration difference between the inner and outer environment of the plant, we were removing, for a certain period of time before the experiment proper, the grains with endosperm from the experimental plants. In the first experiment this period was 72 h and within it the roots of one half of the plants were submerged in water, the roots of the other half being placed in the solution of NaHU at a concentration of 100 mg l^{-1}. Following this preparatory phase the plants were transferred to the KH_2PO_4 solution with a phosphorus concentration of 10.8 mg l^{-1}. The plants were placed each time by 5 in a 200 ml solution so that to each of them 432 μg of phosphorus (P) was available in 40 ml of the solution. In 50% instances sodium humate with a concentration of 100 mg l^{-1} was added to this solution; thus the whole experiment proceeded in 4 variants with the following combination of solutions during the preparatory phase of starvation and the experiment itself:

Variants	Preparatory phase	Experiment
1	water	P + water
2	water	P + NaHU
3	NaHU	P + water
4	NaHU	P + NaHU

The results of the first experiment are set out in Table 1 and graphically represented in per cent of the first variant (water — P + water) in Fig. 1.

This experiment showed that humate slows down the uptake of phosphate ions in maize plants with their endosperm removed and that the slow-down rate is greater if the humate is acting already before supplying the phosphate to the medium; if the humate has been incorporated in the medium simultaneously with the phosphate, the effect of the former will be delayed.

TABLE 1

The amount of phosphorus [μg] taken up by one maize plant free of endosperm in 1, 4 and 5 days, fresh mass of one plant after 5 days of cultivation and the amount of phosphorus taken up during the 5-day experiment per 1 g of root fresh mass

Variant		Fresh mas of 1 plant [mg]		Amount of phosphorus [μg] taken up			
Preliminary phase of starvation	Experiment KH_2PO_4 +			by one plant in			per 1 g of root fresh mass in
		top	root	1 d	4 d	5 d	5 d
water	water	506	236	224	260	312	1 322
water	NaHU	500	306	226	244	269	879
NaHU	water	490	338	200	230	254	751
NaHU	NaHU	522	302	162	168	206	682

However, the humate increased the fresh mass of the plant, primarily of its roots, and therefore the phosphorus uptake suppression is greater when the P absorption rate is related to the fresh mass of the roots.

Now it was necessary to verify the results obtained in some detail, especially with regard to the effects of the starvation period, changes in medium concentration during the experiment and the time course of the experiment. This was the aim of the second experiment whose design was similar to the first one, the plants, however, being subjected to starvation partly for 48 h, partly for 72 h. The humate concentration was 100 mg l^{-1}; with the plants

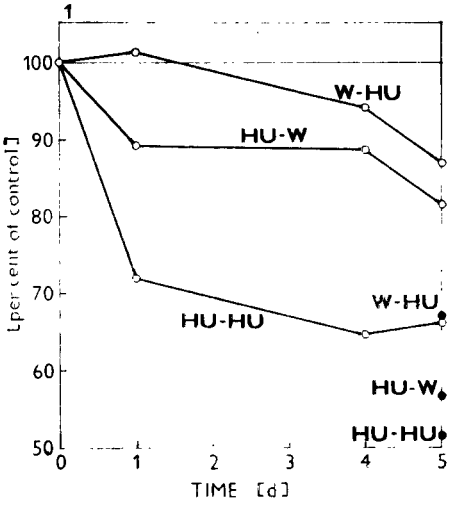

Fig. 1. Decline of phosphorus uptake by 1 maize plant devoid of endosperm during 5 days (abscissa) of cultivation due to sodium humate. The decline values in individual variants (denotations of curves) are expressed in per cent (ordinate) of the variant water — P + water (control). Suppression of phosphorus uptake related to root fresh mass at the end of the 5th day is marked by closed circles.

TABLE 2

The amount of phosphorus [μg] taken up by 1 maize plant free of endosperm in 1, 2, 3, 4, 5 and 6 days, fresh mass of one plant after 6 days of cultivation and the amount of phosphorus taken up per 1 g of root fresh mass after the 6-day experiment

Variant		Fresh mass of 1 plant [mg]		Amount of phosphorus [μg] taken up						
				by one plant in						per 1 g of root fresh mass in
Preliminary phase of starvation	Experiment KH_2PO_4 +	top	root	1 d	2 d	3 d	4 d	5 d	6 d	6 d
water	water $\bar{x}$	614	182	105	190	242	299	361	414	2 306
48 h	$s_{\bar{x}}$	38.30	15.86	4.40	8.60	11.92	15.21	20.62	25.10	201.36
NaHU	NaHU $\bar{x}$	596	347	74	136	163	193	220	234	672
48 h	$s_{\bar{x}}$	32.03	36.61	6.31	12.70	18.49	24.01	28.05	32.18	47.96
water	water $\bar{x}$	593	202	84	140	204	273	320	394	1 955
72 h	$s_{\bar{x}}$	37.37	13.02	6.46	10.94	16.86	21.30	24.30	27.57	62.75
NaHU	NaHU $\bar{x}$	562	298	44	56	72	90	95	114	384
72 h	$s_{\bar{x}}$	18.54	6.79	3.65	8.13	12.36	15.32	16.81	20.76	69.57

starving in the medium with NaHU it was kept at the same concentration also during the experiment with 8.8 mg l^{-1} of phosphorus added as KH_2PO_4 to the culture medium. The plants were placed each time by 5 in 200 ml of this solution so as to provide each of them with 352 μg of phosphorus in 40 ml of solution. In contrast to the first experiment these solutions were

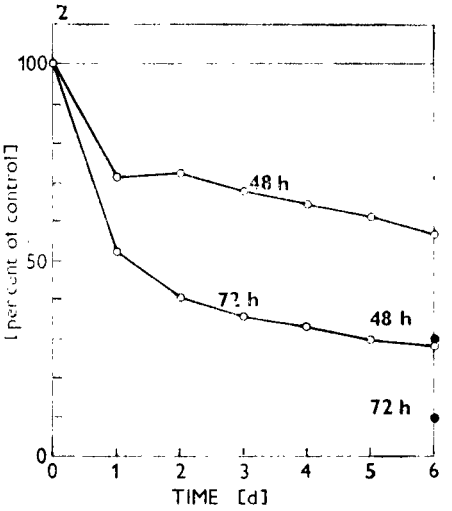

Fig. 2. Decline of phosphorus uptake by 1 maize plant devoid of endosperm during 6 days (abscissa) of cultivation due to sodium humate. The decline values in both humate variants are expressed in per cent (ordinate) of the variant water — P + water (control). Phosphorus uptake suppression related to root fresh mass at the end of the 6th day is marked by closed circles.

replaced daily, having the original concentration. The whole experiment was thus performed in 4 variants with the following combinations of conditions in the preparatory phase of starvation and the experiment proper:

Variants	Preparatory phase	Experiment
1	water, 48 h	P + water
2	NaHU, 48 h	P + NaHU
3	water, 72 h	P + water
4	NaHU, 72 h	P + NaHU

The results of this experiment are set out in Table 2 and graphically presented in Fig. 2. They show that a prolongation of the time of preliminary starvation in the variant water — P + water slows down initially the rate of the subsequent phosphorus uptake. Later this rate becomes equal to that in the 48 h variant. The total amount of phosphorus uptake is decreased in this variant due to the prolonged time, but not significantly. The presence of NaHU during starvation and the subsequent taking up of the phosphate produce a slowing down of phosphorus uptake and a decrease in the total amount taken up by the plant. This decrease is significantly greater than that following the prolongation of the starvation period, and *vice versa*. Humate increases the fresh mass of the root highly significantly; the increase

TABLE 3

The amount of phosphorus [μg] taken up by 1 maize plant free of endosperm in 1, 2 and 3 d of cultivation, fresh mass and dry mass of one plant after 3 d and the amount of phosphorus taken up per 1 g of root fresh mass during the 3-d experiment

Variant		Fresh mass of 1 plant [mg]		Dry mass of 1 plant [mg]		Amount of phosphorus [μg] taken up			
						by one plant in			per 1 g of root fresh mass in
Preliminary phase of starvation Ca^{2+}, Mg^{2+} +	Experiment KH_2PO_4 +	top	root	top	root	1 d	2 d	3 d	3 d
water	water $\bar{x}$	360	215	146	62	92	146	184	872
	$s_{\bar{x}}$	11.05	8.66	4.84	3.26	3.59	3.07	5.76	61.14
NaHU	NaHU $\bar{x}$	352	219	151	72	71	115	150	689
	$s_{\bar{x}}$	8.39	7.46	4.72	2.09	4.64	5.10	6.10	25.05
% of variant 1		97.8	101.8	103.4	116.1*	77.2**	78.8**	81.5**	79.0*

* — statistically significant difference;
** — statistically highly significant difference.

of the whole plant fresh mass is not significant. The increase is greater with a shorter starvation time than with a longer one. With regard to the relevant variants without humate, NaHU decreases significantly the amount of phosphorus absorbed per unit of the root fresh mass. This figure is also significantly lower in the variant with NaHU and a longer starvation time. The two mineral variants do not differ significantly in phosphorus uptake per 1 g fresh mass of the root.

Considering other causes that might contribute to a decline in phosphate uptake observed in our experiments, we directed our attention to the osmotic properties of the medium in which the preliminary phase of starving takes place. In the third experiment we replaced water, serving up to them as the medium, by a solution containing Ca^{2+} and Mg^{2+} ions (see the section Material and Method), used for this purpose, for example by KUMMEROVÁ (1983). The concentration of sodium humate was lowered to 50 mg l^{-1}. The number of vessels in the variant was increased to 10; the solution used contained 9.25 mg phosphorus per 1 litre so that to each plant 370 μg of phosphorus was available in 40 ml of the solution. The experiment featured only two variants differing in the presence of NaHU which was added in the relevant variants at the same concentration to the Ca^{2+} and Mg^{2+} solution during the preparatory phase of starvation:

Variant	Preparatory phase	Experiment
1	water, Ca^{2+}, Mg^{2+}	P + water
2	NaHU, Ca^{2+}, Mg^{2+}	P + NaHU

The results of this experiment are shown in Table 3. It follows from the table that the effect of humate in this experiment, compared with that in the previous experiments, was rather moderate; nevertheless, even here the decrease in absorption rate in the humate variant amounts to about 20% and is highly significant. Worth noting is the altogether inappreciable lessening of this decrease on the 2nd and 3rd days of the experiment, which is apparently connected with the lower humate concentration used.

Adopting Flaig's idea of a more effective action of humate in extreme conditions of the medium, we kept the pH of the medium at 4.8 in the first experiments during the investigation of phosphorus uptake. Now, it was necessary to find out how such a concentration that is usually considered to be the optimum one for the growth of maize, influences the observed effect of sodium humate. Therefore, to conclude the study, an experiment was carried out in which plants without endosperm and with a humate concentration of 50 mg l^{-1} were used again. The absorption proceeded in a solution containing 10 mg l^{-1} of phosphorus. There were 5 vessels with 200 ml of solution and 5 maize plants in each variant. Thus to each plant 400 μg of phosphorus was available in 40 ml. The pH of the solutions was adjusted to 6.3. The experiment had three following variants:

Variant	Preparatory phase	Experiment
1	water	water, pH 4.8
2	water	water, pH 6.3
3	water	NaHU, pH 6.3

The results of the experiment are summarized in Table 4. The experiment fully confirmed the results obtained in the previous experiments. Again the

TABLE 4

The amount of phosphorus [μg] taken up by one maize plant free of endosperm during 2 d, fresh mass and dry mass of one plant after 2 d of cultivation and the amount of phosphorus taken up per 1 g of root fresh mass after two days of experiment at pH = 4.8 and 6.3

Variant			Fresh mass of 1 plant [mg]		Dry mass of 1 plant [mg]		Amount of phosphorus [μg] taken up			
							by one plant in			per 1 g of root fresh mass in
Preliminary phase of starvation	Experiment KH_2PO_4 +		top	root	top	root	1 d	2 d	2nd day	2 d
water	water	$\bar{x}$	393	164	29	10	150	149	− 1	911
	pH 4.8	$s_{\bar{x}}$	9.99	4.66	0.56	0.86	2.23	8.17		19.73
water	water	$\bar{x}$	369	173	28	9	129	110	−19	636
	pH 6.3	$s_{\bar{x}}$	12.72	5.28	0.95	0.69	1.94	3.71		24.66
% of variant 1			93.9	105.5	96.6	90.0	86.0**	73.8**		69.8**
NaHU	NaHU	$\bar{x}$	318	167	23	9	130	85	−45	515
	pH 6.3	$s_{\bar{x}}$	12.84	8.19	1.13	0.54	2.04	2.86		17.10
% of variant 2			86.2*	96.5	82.1**	100	100.8	77.3**		81.0**

* — statistically significant difference;
** — statistically highly significant difference.

inhibitory effect of humate on phosphorus absorption was revealed, even at pH = 6.3. Of extraordinary interest are the figures characterizing the uptake of phosphorus in one or two days (Table 4, columns 6 and 7). If the amount of phosphorus taken up in two days is less than the amount absorbed on the first day, this means that the phosphate received the first day of the experiment is in turn released to the medium on the second day. Humate interacting with the acidity of the medium can apparently exercise a very marked influence on the permeability of the plasmalemma for phosphorus to the degree of reaching a state when this ion escapes from the cell after the concentration decline, if not even actively discharged. The results suggest that humate is involved in transport mechanisms of the plasma membranes and in this connection its action cannot be considered without taking account of the energy metabolism of the cell and the whole plant. But in that case it is apparently necessary to extend the studies so far carried out to plants with a high level of energy functions, hence to the plants with remaining stores of endosperm. For the same reason also the interaction of humate with nitrogen management cannot be excluded from experimental projects. We will present some results of studying the effect of humate on phosphorus uptake in terms of this last suggestion in our further reports.

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