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Changes in distribution of water in plants as a result of the absorbtion of nitrates or ammonium salts

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INTRODUCTION

In the recent years the hypothesis of the active absorbtion of water (in a metabolic sense) has been supported by many investigators who have stressed the role of the energy released in processes of biological oxydation in the absorbtion and transport of water. Rufelt (1959) has found that in wheat seedlings the transport of water in processes of transpiration and of roots pressure is blocked by some respiratory inhibitors such as NaN_3, DIECA, 2,4-DNP. Gumiński, Badura and Buczek (1959) have shown experimentally that the absorbtion of water in transpiring tomato and maize seedlings is correlated with the respiration of roots. Inhibitors of respiration and of phosphorylation used by the authors (KCN, DIECA, Malachite Green and 2,4-DNP) have distinctly blocked the absorbtion of water. From the experiment in which DNP has been applied it results that the energy released in a process of respiration, before being utilized by the process of absorbtion, had to be previously stored in form of high energetic phosphorus bonds.

It has been proved that some mineral salts, mostly nitrates, "stimulate" the increase of the absorbtion of water in plants. The studies on this problem have been carried out by Poskuta (1961) who measured the volume of juice leaking out from the cut off roots of maize. He has stated that nitrates show greater effect on the amount of leaking out juice than other forms of nitrogen's compounds. This relation has been distinctly observed in non aerated media.

The contribution of nitrates to the active absorbtion of water will be clear if we bear in mind that NO_3 ions are able to compensate the deficiency of air in the environment of roots.

This property of NO_3 ions has ben experimentally shown by Arnon (1937), Stabrowska (1957), and Gumiński et al. (1957) in the paper on respiration of tomato and maize roots grown in anaerobic conditions in presence of either nitrates or ammonium salts. The experiments carried out by these investigators have shown that in poorly aerated medium nitrates are able to compensate the deficiency of oxygen in roots of some plants.

Hence, if we assume that, according to the experiments mentioned above the absorbtion of water and its transport are to some extent correlated with the oxygen metabolism then we should expect that the so-called "nitrate's respiration" may become a very important factor in the absorbtion of water by roots of some plants, especially in poorly aerated media.

Thus a series of experiments has been carried out in order to explain whether the oxydized forms of nitrogen (nitrates) compared with the reduced salts of nitrogen (ammonium salts) would increase the absorbtion of water and its transpiration in deficiency of oxygen.

We decided moreover to investigate the simultaneous effect of nitrates and ammonium salts on the transpiration of plants. Such a scheme of experiments has enable us to compare the effects of nitrates and ammonium salts on the water distribution in plants with respect to the amount of oxygen in medium (on which the roots have been grown).

THE DESCRIPTION OF EXPERIMENTS

Experiment I

The distribution of water in 14 days old tomato and flax seedlings in relation to the absorbtion of either nitrate or ammonium-ions from incomplete media

Methods

14 days old tomato and flax seedlings have been the object of experiments. The seeds were washed out in distilled water and sown into clean quartz sand. The germination took place in a green house in 21—23°C. Afterwards the plants were picked out from the sand and the roots were washed out in distilled water, then 4 alike specimens of plants have been taken at a time for each separated experiment.

The seedlings prepared were put for 24 hours in destilled water. The next day the roots of plants were carefully dried out on the blotting paper, and the whole plants were quickly weighed on torsion scale with an accuracy up to 1 mg. In this way the fresh weight of plants has been determined. Then the plants have been put into potometers filled with solution of suitable salts.

In this experiment some incomplete media have been used (solutions of some mineral salts). It enabled us to investigate the direct effect of either nitrate or ammonium ions on the distribution of water in plants. The solution of ammonium salts or nitrates has been prepared by completing the basic medium with the solution of suitable salts, the nitrogen's content being equal to 0,278 mg/l. The possible oscillations of pH have been compensated by 0,1n KOH. The table below shows the amounts of suitable salts in solutions used for the experiment.

	Control medium	Solution with nitrates	Solution with ammonium salts	Distilled water
KCl	0.0307 M	0.0307 M	0.0307 M	-
$CaCl_2$	0.0099 M	-	0.0099 M	-
$Ca/NO_3/_2$	-	0.0099 M	-	-
NH_4Cl	-	-	0.0199 M	-
pH	6.48	6.68	6.72	6.30
Anion/Kation	1.12	1.45	1.25	-

Test-tubes have been used as potometers. The test-tubes were calibrated with an accuracy equal to 0,5 ml. The plants were on the common corks tightend with cotton and vaseline in order to prevent the solution to evaporate, and to make impossible the exchange of gases between the medium and the air. In this way the conditions required for poorly aerated medium have been obtained. The tightening was, however, not so accurate, as to cause the hypopressure due to transpiration.

The investigatios have been carried out in a phototermostate with constant light equal to 9300 Lx, constant temperature 24—25°C, and constant relative humidity equal to 65%. Phototermostate was lighted with electric lamps (bulbs). The intensity of illumination has been measured by standard luxometer of Carl Zeiss, Jena. The relative humidity of the air has been measured by hair higrometer, the type of Lambert's Polymeter.

The experiment lasted for three days. The measurements have been taken in 24 h. intervals. The absorbtion of water was measured on the test-tube scale. The transpired water has been determined by weighing the test-tubes with plants on an analytical balance. The results obtained for absorbed and transpired water have been calculated per 1 gram of fresh weight per 24 hours for each variant of the replication. The average error μ has been found from the following formula:

$$\mu = \pm \sqrt{\frac{\Sigma(f)^2}{n}},$$

where f — denotes the difference between each separate measurement and the arithmetical mean,

and n — denotes the number of experiments.

The results of the experiment I

Tomato-plants. Diagrams 1a and 1b represent the intensity of the absorbtion of water, and the intensity of transpiration, respectively. We can see that the absorbtion of water and its transpiration depend on the form of nitrogen's compound supplied to the roots. Nitra-

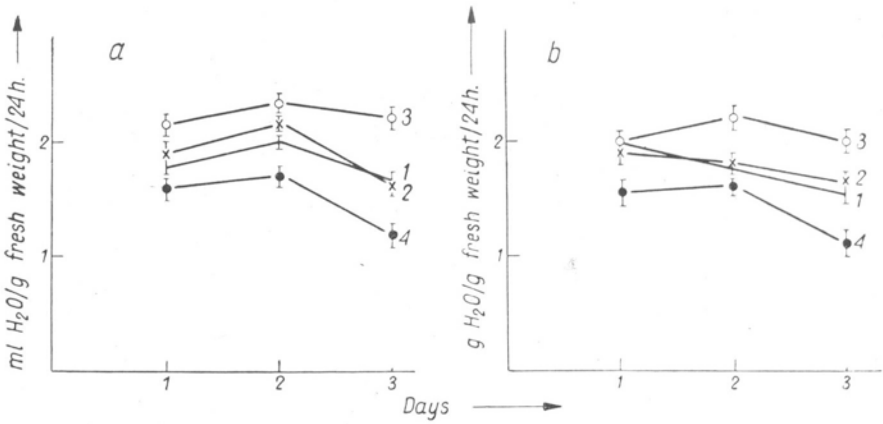

Diagram 1. Tomatoes.

a — absorbtion of water; *b* — transpiration; *1* — distilled water; *2* — $KCl + CaCl_2$; *3* — $KCl + Na(NO_3)_2$; *4* — $KCl + CaCl_2 + NH_4Cl$.

tes compared with the solution of $KCl + CaCl_2$ and with distilled water have had an advantageous effect on both processes. Yet the solution of ammonium salts compared with the control solution has distinctly inhibited the absorbtion and transpiration of water. In this experiment

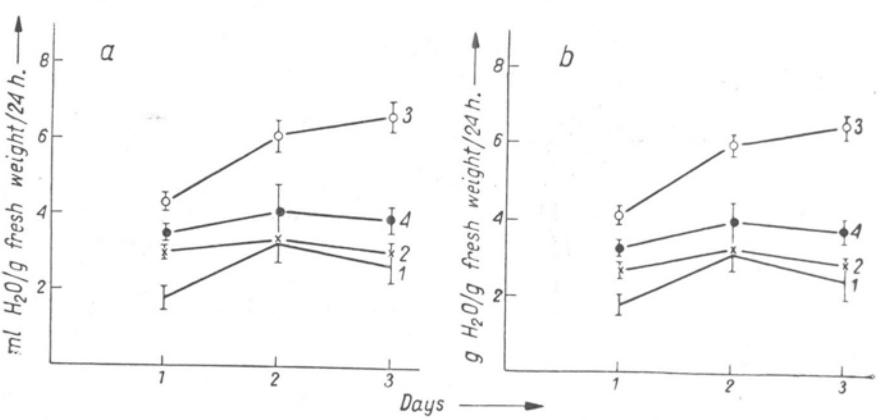

Diagram 2. Flax.

a — absorbtion of water; *b* — transpiration; *1* — distilled water; *2* — $KCl + CaCl_2$; *3* — $KCl + Ca(NO_3)_2$; *4* — $KCl + CaCl_2 + NH_4Cl$.

Table 1

Total amounts of the absorbed and transpired water and the water balance. Incomplete media.

Plants / Solution	Tomatoes						Flax					
	Initial fresh weight g	Amount of absorbed water		Amount of transpired water		Water balance	Initial fresh weight g	Amount of absorbed water		Amount of transpired water		Water balance
		ml /g of initial fresh weight	%	g /g of initial fresh weight	%			ml /g of initial fresh weight	%	g /g of initial fresh weight	%	
H_2O dest.	0.161	9.03	102	8.84	103	+ 0.19	0.226	7.77	84	7.47	83	+ 0.30
$KCl + CaCl_2$	0.158	8.83	100	8.57	100	+ 0.26	0.218	9.30	100	8.91	100	+ 0.39
$KCl + Ca/NO_3/_2$	0.159	16.63	188	16.18	189	+ 0.45	0.192	16.94	182	16.59	186	+ 0.35
$KCl + CaCl_2 + NH_4Cl$	0.170	5.91	67	5.49	64	+ 0.42	0.201	11.55	124	11.24	126	+ 0.31

the effect of $KCl + CaCl_2$ solution on the water distribution in tomato plants has shown no difference with the effect of distilled water.

F l a x. The results are given in Diagram 2. Nitrates have similarly increased the absorbtion of water (diagram 2a) and the transpiration (diagram 2b), in all days of measurements. The results are statistically significant. Yet ammonium salts have not blocked the distribution of water in seedlings of flax and compared with the control solution ($KCl + CaCl_2$) have even increased both processes.

Table 1 shows percentages of absorbed and transpired water in tomato and flax seedlings for the whole experiment (3 days), the solution of $KCl + CaCl_2$ being assumed as 100%.

From the above table we can easily see that plants that have absorbed nitrates show an increased transpiration and absorbtion of water. The relative quantity of the absorbed water has increased in tomatoes by 88% and in flax by 82% compared with plants growing on control solution. Similar situation may be observed in the process of transpiration. On the other hand, in tomatoes ammonium salts have blocked the transpiration by 33% and the absorbtion of water by 36%. In flax ammonium salts compared with the control solution have not inhibited both processes, however, both absorbtion and transpiration of water have been considerably less intensive than in plants grown on nitrates.

Experiment II

The distribution of water in 14 days old tomato seedlings in relation to the absorbtion of either nitrates or ammonium salts from complete media.

Methods

In this experiment the absorbtion and transpiration of water in relation to the absorbtion of either nitrates or ammonium salts from complete media have been investigated. Results have been calculated for the initial fresh weight of plants and for the dry matter. Both fresh and dry weights have been determined each day while liquidating a part of the plants. From the obtained data the amounts of the absorbed and transpired water, and the water balance have been calculated. The water balance has been also determined from the increases of fresh weight of the investigated plants. In this way it was possible to check the methods used in previous experiments.

Experimental plants consisted of 14 days old tomato seedlings germinated in quartz sand. The germination and growth took place in green house. After 10 days the plants have been picked out from the sand, then placed on nylon net-work. Two plants at a time have been carefully chosen for each replication of a given combination of salts, and after having determined the fresh weight (in a way described in experiment I) we put the plants into potometers filled with suitable media. The potometers being the same as in experiment I.

Solutions of mineral salts have been prepared with distilled water from the basic medium (Truffaut and Humpe 1938, 1939) with the following compounds given in grams per one liter: $(NH_4)_2HPO_4$ — 0,124 g., $MgSO_4$ — 0,248 g., KNO_3 — 0,568 g., $Ca(NO_3)_2$ — 0,717 g., $FeCl_3$ — 0,112 g., KCl — 0,141 g.

From the basic medium the following solutions of mineral salts have been done:

Medium without nitrogen: KH_2PO_4 — 0,127 g, $MgSO_4$ — 0,284 g, KCl — 0,490 g, $CaCO_3$ — 0,412 g, $FeCl_3$ — 0,112 g.

Medium with nitrates: $Na_2HPO_4 \cdot 12H_2O$ — 0,335 g, $MgSO_4$ — 0,284 g, KNO_3 — 0,759 g, $Ca(NO_3)_2$ — 0,711 g, $FeCl_3$ — 0,112 g.

Medium with ammonium salts: $(NH_4)_2SO_4$ — 0,943 g, $(NH_4)_2HPO_4$ — 0,124 g, $MgSO_4$ — 0,284 g, K_2SO_4 — 0,653 g, $CaCO_3$ — 0,412 g, $FeCl_3$ — 0,112 g.

To each medium microelements from A—Z Hoagland's solution have been added. The possible oscillations of pH have been compensated by 0,1n KOH and 0,1n H_3PO_4 up to pH = 6,8.

The conditions of the experiment and the measurements have been identical as in experiment I.

Each variant of the experiment consisted of ten repeated experiments (replications), and the results have been computed from the aritmetical mean obtained from ten replications. Results have been calculated per one gram of the initial fresh weight per 24 hours, and per 100 grams of dry matter, per 24 hours for each replication of a given combination of salts. In order to determine the dry matter each day a part of plants has been dried in 105°C, and weighed. At the same time the increases of fresh weight have been weighed in order to determine directly the water balance. The average error has been calculated from the formula given before. The experiment lasted for 3 days.

Results of the experiment II

On the contrary to the preceding experiments the seedlings of tomatoes have been grown on complete media. Results referring to the absorbtion and transpiration of water are given in Diagram 3. In this experiment a positive effect of NO_3 ions on the absorbtion and transpiration of water is distinctly seen. Results compared with data obtained for control plants (growing on medium without nitrogen) are beyond limits of error. The situation is almost the same if the results are calculated per dry weight (Diagram 4).

Yet ammonium salts absorbed by tomato seedlings from complete media, compared with control plants, have blocked neither absorbtion nor the transpiration of water, although these processes are remarkably less intensive than in combination with nitrates.

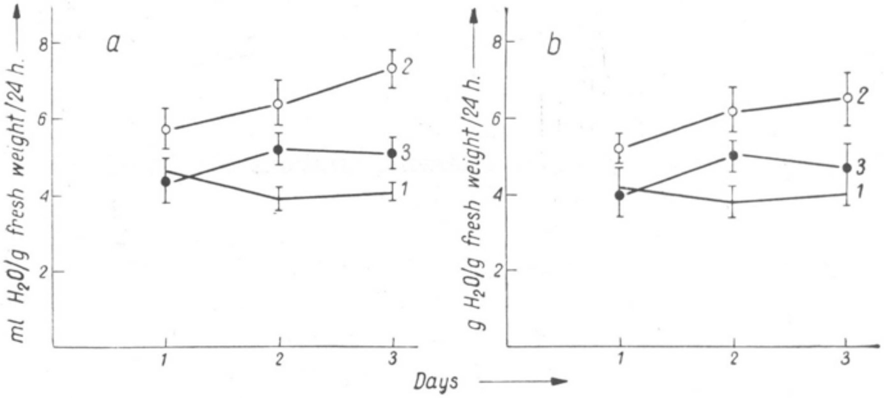

Diagram 3. Tomatoes.

a — absorbtion of water; *b* — transpiration; *1* — medium without nitrogen; *2* — medium with nitrates; *3* — medium with ammonium salts.

The results discussed above are shown in Table 2. In this table the percentages of total amounts of absorbed and transpired water are given for the whole experiment (3 days). Solution without nitrogen is assumed as 100%. It is evident that the stimulating effect of NO_3 ions on the absorbtion and transpiration of water and a slight increase of

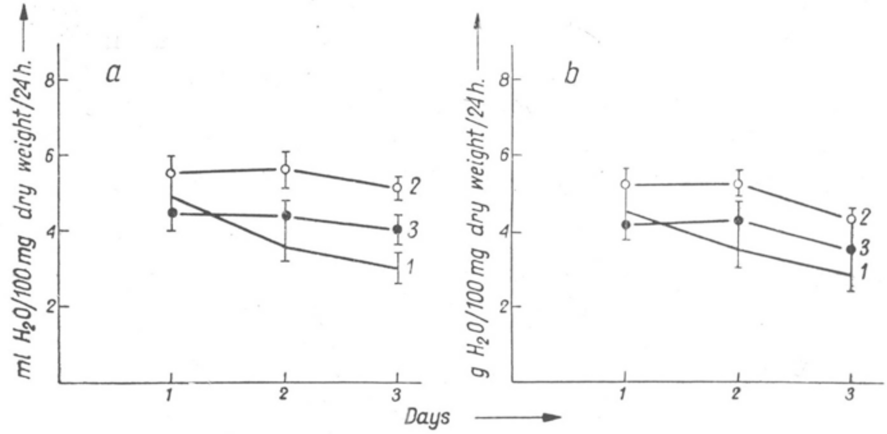

Diagram 4. Tomatoes.

a — absorbtion of water; *b* — transpiration (calculated per 100 mg. of dry matter); *1* — medium without nitrogen; *2* — medium with nitrates; *3* — medium with ammonium salts.

Table 2

Total amounts of the absorbed and transpired water and the water balance. In complete media.

The plant	Tomatoes										
Way of calculation	Per one gram of initial fresh weight						Per 100 mg. of dry weight				
Medium	Initial fresh weight g	Amount of absorbed water		Amount of transpired water		Water balance	Amount of absorbed water		Amount of transpired water		Water balance
		ml /g of initial fresh weight	%	g /g of initial fresh weight	%		ml /100mg of dry weight	%	g /100mg of dry weight	%	
Without nitrogen	0.154	12.67	100	11.24	100	+ 0.73	11.53	100	10.85	100	+ 0.68
With nitrates	0.143	19.48	154	17.93	150	+ 1.55	16.32	141	14.77	136	+ 1.55
With ammonium salts	0.138	14.69	116	13.79	115	+ 0.90	12.96	112	11.97	110	+ 0.99

T a b l e 3

Fresh and dry weights in the experiment II.

The plant	Tomatoes								
Days	0		1		2		3		Water balance after 3 days
Medium	Initial fresh weight mg	Initial dry weight mg	Fresh weight mg	Dry weight mg	Fresh weight mg	Dry weight mg	Fresh weight mg	Dry weight mg	
Without nitrogen			161	14.9	188	17.1	199	18.8	+ 72
With nitrates	121	12.8	173	15.0	190	18.3	278	22.7	+ 147
With ammonium salts			149	13.5	163	15.5	210	17.5	+ 84

T a b l e 4

The water balance in percentages for the Experiment II.

Medium	Per one gram of fresh weight	%	Per 100 mg of dry weight	%	Determined directly mg	%
Without nitrogen	0.73	100	0.68	100	72	100
With nitrates	1.55	212	1.55	228	147	204
With ammonium salts	0.90	123	0.99	145	84	116

both processes due to ammonium salts do not depend on the way of calculation (whether initial fresh weight or dry matter have been the starting point for the calculations). The water balance found from the difference between the absorbed and transpired water does not depend on the way of computation. Table 3 shows the increases of dry weight. If we compare the relative percentages of water balances obtained for each separate combination of salts (solution without nitrogen being assumed to be equal to 100%) we get similar results (Table 4). There is always a positive effect of NO_3 ions and less efficient one of NH_4 ions as compared with control plants.

Experiment III

The absorbtion and transpiration of water in 4 weeks old tomato and flax seedlings due to the absorbtion of either nitrates or ammonium salts, and to the amount of oxygen in medium

Methods

Big grown up plants have been the object of experiments. They have been placed for the time of experiment in potometers filled with suitable solutions containing different forms of nitrogen. From the control medium nitrogen has been removed, and the missing elements have been substituted by suitable salts. One part of the plants has been placed in potometers with aerated media while the second one have been grown in potometers with non aerated media. On the basis of this experiment we were able to infer whether the „stimulating" effect of nitrates on the transport of water consisted in compensation of oxygen deficiency in medium by oxygen derived from NO_3 ions. The experiment has been carried out on tomato and flax.

Young seedlings germinated in sand have been transferred into procelain pots containing 5 liters of Knop's medium diluted in ratio 2:5. The plant had been growing there for 3 weeks and afterwards they have been removed, and carefully selected considering the volume of roots and the seize of the whole plant. For each replication three plants of equal size have been chosen. The roots were carefully dried on the blotting paper and 3 plants at a time were weighed on torsion scale in order to determine the fresh weight; afterwards they have been placed in potometers filled with suitable solutions.

The chemical composition of each medium have been given in experiment II. Media have been prepared with boiled tap water. The surface of tap water, freshly boiled in flask with long necks was covered with paraffine and cooled to 20°C. Afterwards the solutions of suitable medium have been poured in concentrations required for any given medium.

Two types of potometers have been used: a) the potometer accomodated to measurements of absorbtion and transpiration of water in continuous aeration, and b) the second one accomodated to measurements in non aerated media.

A e r o b i c c o n d i t i o n s. The plants have been put into conic flasks calibrated up to 1 ml. and placed on the cork tightend with cacao butter. There were also two glass tubes in the same cork. One of them was long and reached the bottom of the flask, and the second one was short and stuck a little from below the cork. The second glass tube was connected with U-tube containing 10% H_2SO_4 to absorb the water. The air entering by the longer tube was pressed in by help of a device, commonly used for aeration of aquaria, and was going out by the U-tube.

A n a e r o b i c c o n d i t i o n s. Similarly calibrated flask were filled with medium and the plants were put inside in the following way: The root stele has been wraped with a thick layer of cotton and put into the hole made in usual cork. The cork was put into a flask and its surface was covered with cacao butter. A very slight gas exchange through the cotton was necessary to avoid the hypopressure that could result from transpiration.

The experiments have been carried out in a phototermostat, which allowed to keep constant temperature 24—25°C, constant relative humidity of the air equal to 75%, and a constant intensity of light = 9300 Lux. The plants have been lighted incessently during the whole experiment which lasted for 3 days.

The measurements have been taken in 24 h. intervals. The amount of absorbed water was directly read in mililiters from the flask's scale, and the amount of transpired water was found by weighing apparatusses on an analytical balance. The results for the absorbed and transpired water were calculated per one gram of the initial fresh weight per

24 hours for each replication of the given combination of salts. The final results for each combination were obtained by computing the arithmetical mean from the 5 replications. The average error was found from the konwn formula given before.

Results of the experiment III

P e r i o d i c a l a e r a t i o n. The experiment has been carried out on tomato plants in aerobic and anaerobic conditions. In aerobic conditions the aeration lasted for ten hours. The whole experiment lasted

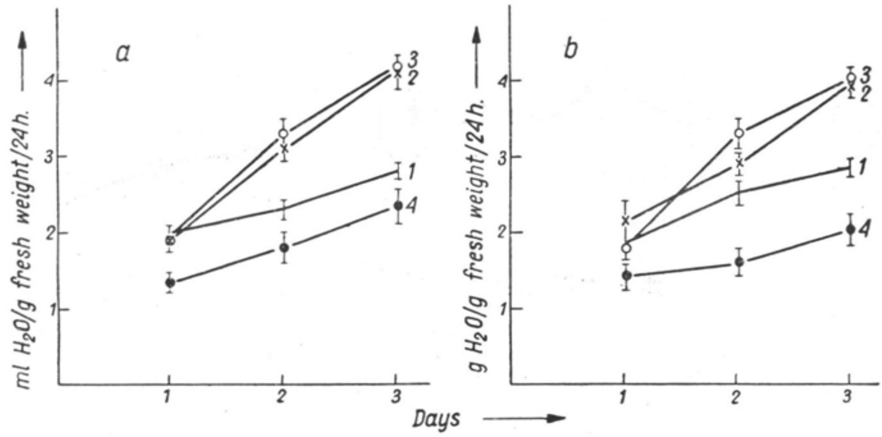

Diagram 5. Tomatoes. Periodic aeration.

a — absorbtion of water; *b* — transpiration; *1* — medium without nitrogen; *2* — medium with nitrates + ammonium salts; *3* — medium with nitrates; *4* — medium with ammonium salts.

for 3 days. In course of the experiment each day apparatusses for aeration have been switched on for ten hours during the remaining 14 hours the medium was not aerated.

The intensity of the absorbtion and transpiration of water in these experiments for aerobic and anaerobic conditions is presented on Diagrams 5 and 6, respectively. From the diagrams it follows that:

1. 24 hours after the experiment had been started there was no increase of the absorbtion of water (Diagram 5-*a*) or of the transpiration (Diagram 5*b*) caused by nitrates in aerobic conditions. The results are within limits of error. Yet the medium with ammonium salts inhibited to some extent the absorbtion of water. The transpiration of plants growing on medium with either nitrates or ammonium salts (Diagram 5-*b*) is less intensive than in plants growing on medium containing nitrates plus ammonium salts.

2. Starting from the second day of measurements, an increased absorbtion and transpiration of water is seen in plants growing on solutions containing nitrates plus ammonium salts, whereas the absorbtion and transpiration of water in plants growing on medium with ammonium salts is distinctly inhibited, when compared with control medium. The same is observed in the next day of measurements.

3. Since the second day of measurements, medium containing nitrates distinctly "stimulates" the absorbtion of water (Diagram 6-*a*) and the transpiration (Diagram 6-*b*) in anaerobic conditions. On the other

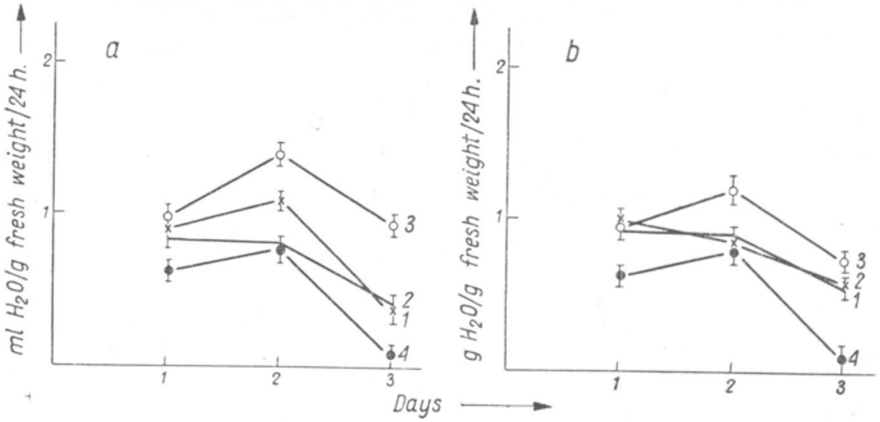

Diagram 6. Tomatoes. Non aerated medium.

a — absorbtion of water; *b* — transpiration; *1* — medium without nitrogen; *2* — medium with nitrates + ammonium salts; *3* — medium with nitrates; *4* — medium with ammonium salts.

hand there is no increased absorbtion and transpiration of water in plants growing on medium containing nitrates plus ammonium salts. The slight differences are within limits of error. (Except the data for the absorbtion of water in the second day of measurements).

4. The absorbtion of water in plants growing on medium containing ammonium salts in anaerobic conditions is always less intensive than in plants growing on the control medium (without nitrogen). Results, except the second day of measurements, are significant.

5. In aerated media the water balance (Table 5) is positive, for all combinations of salts, except for media without nitrogen, in anaerobic conditions where it is positive only for media containing nitrates.

Continuous aeration. Further experiments on tomato and flax have been carried out similarly, but instead of periodical aeration the aeration has been continuous, stopped only for the moments of reading the results.

Results obtained from these experiments carried out on two different species of plants are in general, similar, and in view of the former experiments (with ten hours aeration) seems to be of interest.

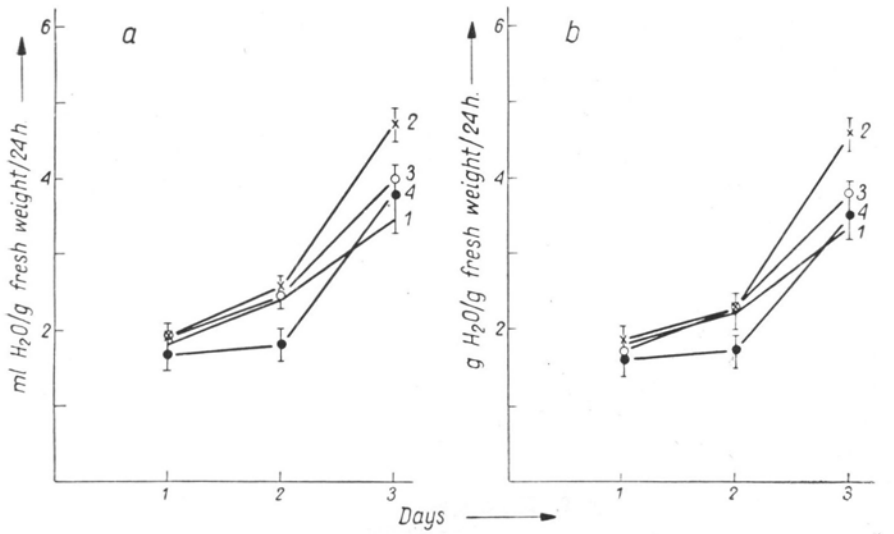

Diagram 7. Tomatoes. Continuous aeration.

a — absorbtion of water; *b* — transpiration; *1* — medium without nitrogen; *2* — medium with nitrates + ammonium salts; *3* — medium with nitrates; *4* — medium with ammonium salts.

Although in comparison with the preceding experiment in anaerobic conditions we do not observe any marked change in tomato plants (Diagram 8) or in flax (Diagram 10) in all combinations of mineral salts, nevertheless if we consider the curves of the absorbtion and of transpiration of water, obtained for continuously aerated media (Dia-

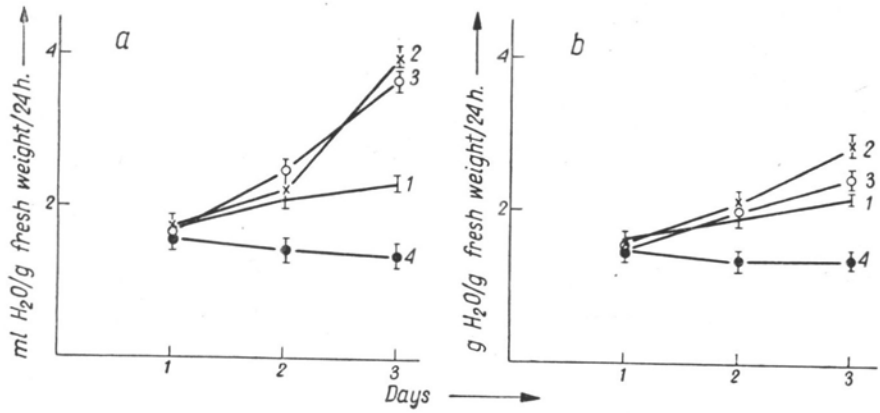

Diagram 8. Tomatoes. Non aerated medium.

a — absorbtion of water; *b* — transpiration; *1* — medium without nitrogen; *2* — medium with nitrates + ammonium salts; *3* — medium with nitrates; *4* — medium with ammonium salts.

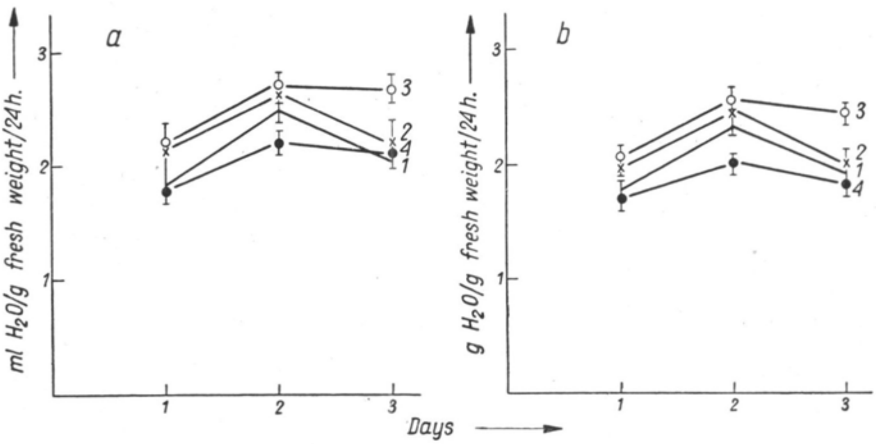

Diagram 9. Flax. Continuous aeration.
a — absorbtion of water; *b* — transpiration. *1* — medium without nitrogen; *2* — medium with nitrates + ammonium salts; *3* — medium with nitrates; *4* — medium with ammonium salts.

gram 7 and 9), than we see striking differences between those results and the results obtained for periodically aerated media (Diagram 5). Those differences are:

1. Continuous aeration has entirely compensated the blockade of the absorbtion and transpiration of water in tomato and flax due to ammonium salts. This compensation has not been observed if the plants were grown on poorly aerated media.

2. An adequate aeration has very distinctly decreased in tomatoes the stimulating effect of nitrates on the absorbtion of water (Diagram 7-*a*) and on transpiration (Diagram 7-*b*), compared with the control

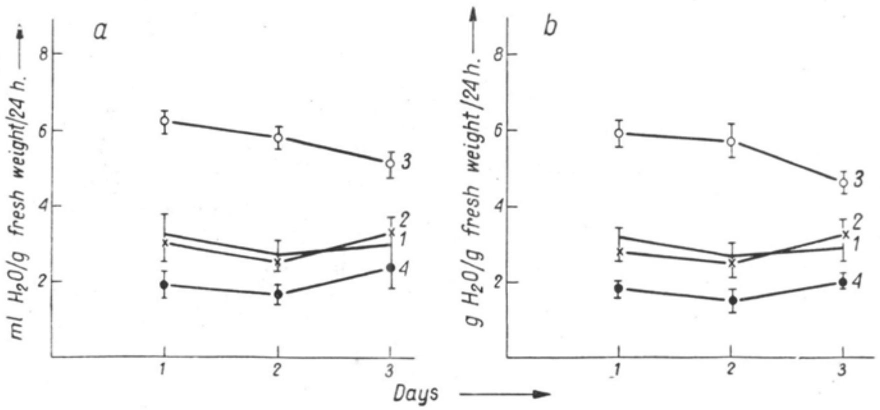

Diagram 10. Flax. Non aerated medium.
a — absorbtion of water; *b* — transpiration; *1* — medium without nitrogen; *2* — medium with nitrates + ammonium salts; *3* — medium with nitrates; *4* — medium with ammonium salts.

Table 5

The total amounts of the absorbed and transpired water and the water balance.

Experiment III

The plant		Tomatoes						Tomatoes						Flax					
Type of the experiment		periodical aeration						continuous aeration						continuous aeration					
General conditions	Medium	initial fresh weight g	amount of absorbed water ml/g of initial fresh weight	amount of absorbed water %	amount of transpired water g/g of initial fresh weight	amount of transpired water %	water balance	initial fresh weight g	amount of absorbed water ml/g of initial fresh weight	amount of absorbed water %	amount of transpired water g/g of initial fresh weight	amount of transpired water %	water balance	initial fresh weight g	amount of absorbed water ml/g of initial fresh weight	amount of absorbed water %	amount of transpired water g/g of initial fresh weight	amount of transpired water %	water balance
Aerated medium	Without nitrogen	2.109	7.06	100	7.21	100	- 0.15	1.604	7.67	100	7.36	100	+ 0.21	1.481	6.38	100	6.02	100	+ 0.36
	With nitrates + ammonium salts	1.964	9.12	129	8.99	125	+ 0.14	1.470	9.14	117	8.70	118	+ 0.44	1.609	6.97	109	6.45	107	+ 0.52
	With nitrates	2.111	9.40	133	9.06	126	+ 0.34	1.904	8.35	109	7.80	106	+ 0.55	1.690	7.59	119	7.07	117	+ 0.52
	With ammonium salts	2.194	5.48	78	5.14	72	+ 0.34	1.909	7.29	95	6.80	92	+ 0.49	1.722	6.09	95	5.54	92	+ 0.55
Non-aerated medium	Without nitrogen	2.518	2.04	100	2.39	100	- 0.35	1.435	6.07	100	5.78	100	+ 0.29	1.714	5.43	100	5.30	100	+ 0.13
	With nitrates + ammonium salts	2.191	2.36	116	2.44	102	- 0.08	1.577	7.70	127	7.63	132	+ 0.07	1.625	5.69	106	5.44	103	+ 0.25
	With nitrates	2.168	3.30	162	2.89	121	+ 0.41	1.692	7.32	120	6.94	120	+ 0.38	1.755	6.70	123	6.22	117	+ 0.48
	With ammonium salts	2.279	1.52	75	1.56	65	- 0.04	1.636	4.42	73	4.30	74	+ 0.12	1.626	4.59	85	4.38	83	+ 0.21

experiment. In flax, however, nitrates have to some extent still stimulated the absorbtion of water (Diagram 9*a*).

The relations discussed above are shown in Table 5. The amounts of absorbed and transpired water are given in percentages, values obtained for the combination of salts without nitrogen has been assumed as 100%. The data presented in Table 5 make possible the comparison of results obtained in the described experiments. In deficiency of oxygen (non aerated medium) we observe in all experiments an increased transpiration and absorbtion of water in plants growing on media with nitrates, and a decrease of both processes in plants growing on media with ammonium salts, compared with the control medium. The same is for medium with nitrates plus ammonium salts. Yet the stimulating effect of nitrates and the blocking effect of ammonium salts may be observed only in periodically aerated medium. Those effects disappear in media, aerated continuously.

DISCUSSION OF THE RESULTS

First experiments carried out on incomplete media have already given a hint as to the solution of the general problem, namely whether the nitrates absorbed by the roots of plants growing in deficiency of oxygen would increase the absorbtion of water. It have resulted from the comparison made between the media containing nitrates with the media containing the reduced forms of nitrogen, i.e. — ammonium salts.

A rather good tightening of potometers (test-tubes) and small surface of the water make the free difusion of atmospheric oxygen difficult. Hence we should expect that if the roots of investigated plants lived in bad aerobic conditions the stimulating effect of nitrate ions on the absorbtion and transpiration of water should be distinct.

Further experiments have been carried out on big, grown up plants. The results are given on Diagrams 5, 6, 7, 8, 9 and 10 and on the Table 5. They show that nitrates can considerably increase the absorbtion and transpiration of water (compared with other media) if and only if the plants have been grown on non aerated media or at least have been aerated for very short period of time. It is author's oppinion that this phenomenon is due to oxygen deriving from nitrates and available for the roots plunged in the liquid medium. Although in anaerobic conditions the potometers have not been entirely tightend, and the difusion of air could occur, but the amounts of oxygen obtained by the difusion, compared with amount of oxygen contained in NO_3 ions would be negligeable. We can calculate theoretically that in one liter of water in 25°C about 40 mg. of oxygen can be dissolved, yet one liter of medium with nitrates (applied by author) contained 776 mg of oxygen present in NO_3 ions (thus about 19 times more). On the other hand the absorbtion and transpiration of water in plants growing on media with ammonium salts, in the same anaerobic conditions, are less intensive or even blocked, when compared with control plants. Yet nitrates stimulate both processes. If however the media with ammonium salts were intensively aerated, then these differences entirely disappear.

The above experiments support our hipothesis that in deficiency of oxygen the nitrates may act as the acceptors of hydrogen in the process of biological oxidation, and that this process may probably produce certain amounts of free energy being used in processes of transport of water in plants.

After author's oppinion the following possible interpretations of the described experiments should be considered:

1. The increased absorbtion of water affected by NO_3 ions and the decrease of this process due to NH_4 ions depend on different intensity with which these ions are absorbed and accumulated.

2. The differences in distribution of water due to nitrates and ammonium salts results from changes of cytoplasmatic permeability for water.

3. The process of the absorbtion of water and its transport depend upon free energy obtained in the process of oxidative respiration. Hence the oxidated salts like nitrates, that are able to act as hydrogen's acceptors, compensate at the same time the deficiency of oxygen suffered by roots. That is why in anaerobic conditions we do not observe any decrease of the absorbtion of water if NO_3 ions have been absorbed; yet the relation between the oxidative respiration and the absorbtion and transport of water become very distinct when the roots have absorbed NH_4 ions.

Several authors (Hamner 1936; Postma 1939; Humphries 1951; Pirson and Gollner 1953; Willis and Yemm 1955; Becking 1956) have observed an increased respiration in plants suffering the lack of oxygen if the media have been completed with ammonium salts or nitrates. This increased respiration was found to be due not to the very absorbtion of the salts but to their contribution to protein metabolism. In plants suffering the lack of nitrogen we see an intensive synthesis of organic nitrogen compounds after ammonium salts being added. This phenomenon is accompanied by a decrease of respiration coefficient to the value 0.8 or even lower. (Syrret 1953; Yemm and Folkes 1954; Millbank 1957), and an intensive consumption of ATP (Syrret 1958). Yet the addition of nitrates resulted in an increase of respiration coefficient to 1.7 or higher. This dependence has been found by Warburg and Negelein 1920, Syrret 1955 and Willis and Yemm 1955. According to these authors the nitrates may act as terminal acceptors of hydrogen. This hypothesis has been justified by experiments of Nicholas and Nason 1954, 1955 and Nason 1956. The mentioned authors have isolated an enzymatic system (nitrate reductase) from *Neurospora* and leaves of soybean. In this system DPN, TPN, FAD and molibden ion are active. The system reduces NO_3 ion and oxidizes either DPNH or TPNH.

In our experiments the effect of nitrates or ammonium salts resulting in significantly different absorbtion of water has been distinctly seen in poorly or non-aerated media, i.e. when the compensation ability of NO_3 ions is most distinctly manifested. In experiment with ammonium salts carried out in deficiency of oxygen there is no factor that would effect the reoxidation of the reduced phosphopyridine nucleotides which take part in e.g. the synthesis of glutamic acid from α-ketoglutaric acid and NH_4 ion (Yemm and Folkes 1954). Finally in plants that absorb ammonium salts in anaerobic conditions the balance of energy becomes labile and affects the distribution of water. Yet in absence of oxygen but in presence of nitrate-ions the phosphopyridine nucleotides which (according to Nason 1956) give the electrons onto the nitrate-reductase, and in consequence, onto NO_3 ion, may be oxidized by the reduction of nitrates. Finally, in anaerobic conditions in presence of nitrates the respiration does not decrease and the released energy may by used up in processes of absorbtion and transport of water.

The author has based his hipothesis on Gumiński et al. (1957) experiments referring to the respiration of tomato, maize and wheat roots in diffferent aerobic conditions in presence of either ferric ion or nitrates or sulphates. The mentioned authors have stated that only nitrates are able to compensate the deficiency of oxygen in roots of plants growing on poorly aerated media. This phenomenon has been explained by the fact that NO_3 ion acts as the acceptor of hydrogen released in process of respiration.

From these investigations the author assume that the increased absorbtion and transpiration of water in plants growing on nitrates in poorly aerated media (and the simultaneous blockade of these processes in plants that absorb ammonium salts) depend on the amonut of free energy released in the process of „nitrate respiration".

There is however the question whether the process of absorbtion of water depends upon respiratory processes to such an extent that even the aeration lasting for a short period of time can be unsufficient for the absorbtion of water and perhaps for its transport in plants?

Gumiński, Badura and Buczek (1959) have shown that the absorbtion of water by roots is closely related with the respiration and with free energy. The poisoning of roots by KCN or DIECA has inhibited both respiration and absorbtion of water, yet 2,4-DNP although has not decreased the respiration has markedly inhibited the absorbtion of water. The experiments have shown that the energy released in the process of respiration decides upon the absorbtion of water and that before being used in a process of "water pumping", it must have been stored in form of high energy phosphorus bonds.

The transport and absorbtion of water are closely dependent on the cytoplasmic permeability for water. Hence, we should expect that the effect of various nitrogen salts resulting in changes of the absorbtion and transport of water is due to the above relation. We know, however, that the normal state of cytoplasmic biocolloids is correlated with regular changes of energy, i.e. any change of the respiration results in changes of cytoplasmic permeability. Hence, if there exist any difference in cytoplasmic permeability for water, then it can also be explained by the oxidoreductive effect of nitrates.

The measurements of transpiration given in diagrams show that the intensity of this process is in general equal to the intensity of the absorbtion of water. Thus certain parallelism occuring between the absorbtion and transpiration of water would show that the same factors may affect the transport of water as well in the process of the absorbtion by roots, as in the process of transpiration by leaves. No wonder that the water balance which, as we know, has been defined as a difference between the absorbed and transpired water, depends upon the factors that affects both processes. The factors that in our experiments had affected the transport of water, were different aerobic conditions given for the roots (aerated or non aerated media), and different forms of the absorbed nitrogen. Since all these factors have equally affected the absorbtion and transpiration of water, then a rather close correlation between transpiration and absorbtion of water should be expected. This is true when the absorbtion of water depends upon the transpiration. In this case the intensity of the absorbtion of water depends on permeability of roots' cells for the water flowing through xylems and in some extent on AFS of roots. Consequently we can say that all the factors that increase the permeability of cytoplasm, and decrease the resistence showed to the water flowing through AFS, and the factors that decrease the viscosity of the solution of salts flowing through the xylem, would determine the intensity of the absorbtion of water in the process of transpiration.

However, the obtained results authorize us to the quite opposite interpretation: namely that the transpiration depedns upon the root pressure. The higher root pressure the higher transpiration. All factors that increase the root pressure will at the same time increase the transpiration. It is possible that nitrate-ions, owing to compensation ability of the deficiency of oxygen, are able to increase the absorption of water in the process of root pressure to such an extent that it affects the increase of the transpiration seen in our experiments.

Further and more detailed investigations have been started by the author.

CONCLUSIONS

1. The absorbtion of water by roots and its transpiration by leaves is affected by the absorbtion of different forms of nitrogen: nitrates or ammonium salts.

2. Nitrates increase the absorbtion of water and its transpiration, yet ammonium salts cause a decrease or a blockade of those processes, compared with the control (without nitrogen).

3. A stimulating effect of nitrates and a blocking one of ammonium salts are distinctly seen in poorly aerated media. Both effects disappear if media are aerated adequately.

4. There is a relation between transpiration and the amount of oxygen in medium. The presence of NO_3 ions or good aerobic conditions increase the transpiration, while the absence of nitrates or bad aerobic conditions decrease the transpiration.

5. We can assume a hypothesis that the increased absorbtion and transpiration of water affected by nitrates in anaerobic conditions, is due to the ability of compensation the deficiency of oxygen shown by NO_3 ions.

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