

The Influence of Na-humate on the Respiration of Wheat Roots and Leaves

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Vliv Na-humátu na dýchání kořenů a listů pšenice

Natriumfluorid, monoiodacetát a malonát brzdí, popřípadě stimulují, dýchání kořenů pšenice pěstované 2 až 10 dní v roztoku humátu sodného (100 mg/l) silněji, než dýchání kořenů rostlin pěstovaných ve vodě. Obdobně působí natriumfluorid na dýchání listů. Poměr radioaktivit $C^{14}O_2$ uvolněného z glukosy značené v poloze 1 nebo 6 (C_6/C_1) je průkazně zvýšen u kořenů, nikoli však u listů. Změna tohoto poměru je doprovázena zmenšením celkové radioaktivity $C^{14}O_2$ uvolněného kořeny rostlin ovlivněnými humátem z glukosy specificky i totálně značené. Endogenní respirace (QO_2) kořenů je působením humátu zesílena o 5—30 %, intenzita respirace listů zůstává na stejné úrovni. Růst kořenů do délky je v prostředí s humátem intenzivnější o 20—80 %, růst listů o 5—15 %. Uvedená zjištění vedou k závěru, že v kořenech rostlin pěstovaných v roztoku humátu vzrůstá podíl glykolysy v respiračním metabolismu.

Summary

Sodium fluoride, iodoacetate and malonate inhibit or stimulate the respiration of wheat roots cultivated for 2—10 days in a solution of sodium humate (100 mg/l.) as compared with the respiration of roots cultivated in water. The influence of sodium fluoride on the respiration of leaves is similar. The ratio of the radioactivities of $C^{14}O_2$ from glucose, labelled in the position 1 or 6 (C_6/C_1) is distinctly higher in roots, but not in leaves. The change in this ratio is accompanied by a decrease in the total radioactive $C^{14}O_2$ from roots of plants influenced by humate, from glucose labelled specifically and totally.

Endogenous respiration (QO_2) of roots increased in humate by 5—30%.

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the intensity of leaf respiration remaining at the same level. The longitudinal root growth increased in humate by 20—80%, the growth of leaves by 5—15%. The above statements show that in a humate solution the glycolytic fraction of the respiratory metabolism of roots is increased.

Introduction

It is known that humic acid in solution of 0.01—0.001% has a positive influence on the growth of the root system as well as on the green parts of plants, but it is different from auxines or antiauxines (ŘEŘÁBEK 1960). It increases the intensity of respiration. According to CHRISTEVA (1953, 1955) humous compounds stimulate the reduction of the terminal oxidases, being a substrate for polyphenol oxidase. ŠMÍDOVÁ (1962) found a total increase of the endogenous respiration in the humate treated plants of a wheat root homogenate, as well as a change in activity of some enzymatic systems acting in oxidation-reduction reactions of respiration. The action of Na-humate lowered the activity of succinic dehydrogenase; the inhibition of respiration by malonate was reduced; the inhibition of respiration by the action of products of the Krebs-cycle increased; the activity of cytochrome oxidase, ascorbic acid oxidase, polyphenol oxidase, lipoxidase and flavin enzymes also increased. From these facts the author assumes a change in activity of the pentose cycle and of glycolysis. FLAIG, SCHARER and SCHOLL (1957) found that thymohydrochinon suppressed the activity of sucrose, amylase, phosphatase and aldolase. ČINČEROVÁ (1962) concluded from the higher amount of pentoses, glucose, sucrose and fructose and lower quantities of aminoacids in wheat roots in the stage of the first leaf influenced by humate, that the above changes are caused by a deficiency of nitrogen inhibiting further sugar metabolism namely glycolysis.

FLAIG (1962) studied the effect of compounds originating from the decomposition of straw and lignins and found that they change plant metabolism by uncoupling oxidative phosphorylation from respiration.

The results of most of the above papers dealing with the relation of humate to the energy metabolism of plants cannot clearly demonstrate changes in the activity of different respiratory systems, such as glycolysis and the pentose cycle in the metabolism, because their methods are inadequate. In the present study we are bringing results of our experiments; we have used specifically labelled glucose and respiratory inhibitors, to investigate the role of glycolysis in plants cultivated in humate.

Materials and Methods

For our experiments we used first leaf-blades and root tips 0.5—1.0 cm. long of wheat (*Triticum vulgare* L. cultivar Stupnická jarní), cultivated in Petri dishes with distilled water or with Na salt of humic acid 100 mg./l. purchased from Riedel de Haen A.G. Seelze Hannover. Wheat was germinated for 24 hours in a humate solution or distilled water and later transferred to a cultivation box with 24—27° C temperature and 97% air humidity. A neon light of 1400 Lx was used for 12 hours a day (cf. LUŠTINEC and POKORNÁ 1962).

The measuring of QO_2 (μ l. O_2 /hour/mg. dry weight) and respiration inhibition by sodium fluoride 3×10^{-2} M, by monoiodoacetate 2×10^{-3} M and malonate 5×10^{-3} M in 0.1 M citrate buffer pH 4.5 were made by the direct Warburg method. The measuring of the C_6/C_1 ratio was performed by a method published previously (LUŠTINEC and POKORNÁ 1962). The 50 ml. Erlenmeyer incubation flask contained: 6 ml. 0.033 M phosphate buffer pH 5.3, 100 root tips or 0.5 g dissected leaf-blades, and 1 ml. specifically or totally labelled glucose, showing a radioactivity of 330 μ C and mass of 4 mg.

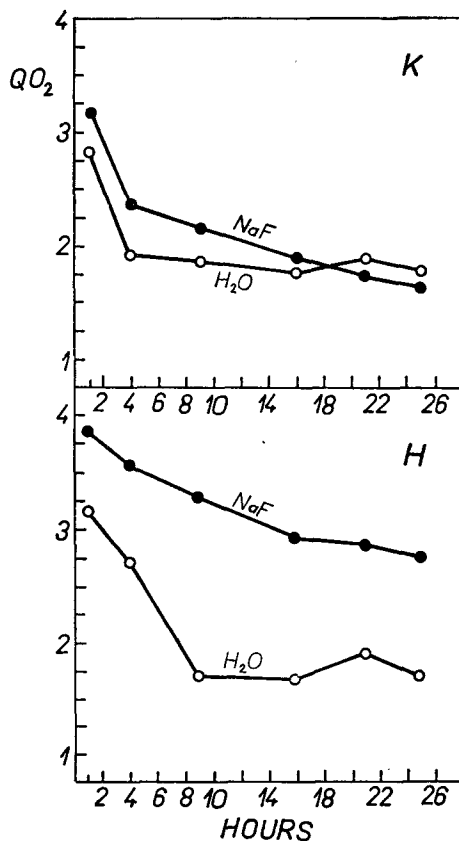
For measuring the radioactivity of $BaC^{14}O_3$ a GM tube with the end window (of the thickness of 1.00—1.05 mg./cm.²) was used. The efficiency of measurement was $1 \mu C = 10^5$ cpm. The values presented here are the results of at least 3 parallel measurements. Each experiment was repeated 3 times.

Results

The tables 1 and 2 show that fluoride, monoiodoacetate and malonate inhibit the respiration of humate-treated plants more than the controls. The intensity of inhibition is not absolutely the same in leaves and roots of the same age, but it is definitely higher in humate-treated plants, even if the oxygen consumption in humate-plants is not increased; it decreases with the age of leaves. In low concentrations of inhibitors or in old tissues the respiration is stimulated; stimulation is higher in humate-treated plants. In some cases the humate-treated plants are still strongly inhibited whereas the control is already slightly stimulated (cp. the experiment with malonate, tab. 2). A similar relation can be found with the long term effect of NaF (Fig. 1). At the beginning the respiration of the humate-treated plants is more intense than in the control. After 16 hours the humate-treated plants are still stimulated, whereas the control is already inhibited by NaF. The ratio C_6/C_1 in roots treated by humate is distinctly higher (tab. 3) mainly due to the decreasing output of $C^{14}O_2$. In leaves the ratio does not change (tab. 4), though the influence of fluoride is the same as in roots. Humate-treated roots show a lower absolute radioactivity of $C^{14}O_2$ produced by roots from specifically and totally labelled glucose (tab. 5).

Fig. 1.

QO_2 (μ l. O_2 /mg. of dry weight during 60 min.) in wheat leaves grown for 5 days in water (K) and sodium humate (H) in relation to time of incubation in water (○) and in sodium fluoride $3 \times 10^{-2}M$ (●).



Tab. 1. The effect of NaF $3 \times 10^{-2}M$ on respiration of wheat-leaves grown in Na-humate 100 mg./l. and in distilled water

Age of leaves in days	Variant	Average length of leaf in cm	$QO_2(H_2O)$	$QO_2(NaF)$	Percentual inhibition - stimulation +
4	K	3.2	6.6	5.8	-12.8
	H	4.0	6.6	5.5	-16.3
6	K	7.8	4.4	4.6	+ 2.9
	H	8.6	4.0	4.7	+ 16.4
8	K	11.9	3.2	4.3	+ 36.0
	H	13.8	3.2	4.9	+ 55.0
10	K	12.7	3.5	4.4	+ 26.9
	H	14.9	3.4	4.6	+ 36.0

Tab. 2. The effect of fluoride, iodoacetate and malonate on respiration of wheat roots

Age of plants in days	Average length of roots in cm		QO ₂		Percentual inhibition (–) or stimulation (+) of oxygen consumption					
					Na-fluoride 3 × 10 ⁻³ M		Na-malonate 5 × 10 ⁻³ M		monoiodoacetate 2 × 10 ⁻⁴ M	
	K	H	K	H	K	H	K	H	K	H
3	2.08	2.58	13.1	14.5	-57.0	-67.0	-31.4	-44.5		
4	2.32	3.52	9.1	13.3	-41.0	-54.0	+ 2.8	-22.0		
6	2.52	3.60	8.5	9.3	-32.0 +12.0*	-44.4 +45.0*			-76.4	-84.1

Na-fluoride 3 × 10⁻³MTab. 3. Production of ¹⁴CO₂ from wheat roots grown in water (K) and in solution of Na-humate (H) 100 mg./l. during two-and four-hour incubation with glucose 1-¹⁴C and glucose-6-¹⁴C

Age of plants in days	Duration of incubation in hours	Variant	Radioactivity of BaC ¹⁴ O ₂ in counts/min./10 mg. dry weight			C ₆ /C ₁		
			G-1-C ¹⁴		G-6-C ¹⁴		G-1+6-C ¹⁴	
			\bar{x}	s \bar{x}	\bar{x}	s \bar{x}		
2	0-2	K	392 ±	5.65	227 ±	1.04	619	0.57
		H	331 ±	2.32	206 ±	1.63	537	0.62
	2-4	K	523 ±	5.70	525 ±	2.19	1048	0.99
		H	357 ±	3.66	424 ±	1.92	781	1.18
4	0-2	K	1097 ±	12.2	146 ±	2.25	1243	0.13
		H	526 ±	4.23	432 ±	1.50	868	0.65
	2-4	K	861 ±	10.8	305 ±	2.22	1166	0.36
		H	657 ±	7.05	392 ±	1.87	1049	0.59

 \bar{x} = means \bar{x} = standard deviationTab. 4. Production of C¹⁴O₂ from wheat leaves of four-day-old plants grown in distilled water and Na-humate 100 mg./l. during two-and four-hours incubation with glucose-1-¹⁴C and glucose-6-¹⁴C

Duration of incubation in hours	Variant	Radioactivity of BaC ¹⁴ O ₂ in counts/min./10 mg. dry weight			C ₆ /C ₁		
		G-1-C ¹⁴		G-6-C ¹⁴		G-1+6-C ¹⁴	
		\bar{x} ±	s \bar{x}	\bar{x} ±	s \bar{x}		
0-2	K	248 ±	13.1	217 ±	3.0	465	0.88
	H	258 ±	6.9	229 ±	10.4	487	0.89
2-4	K	314 ±	17.1	304 ±	9.3	618	0.96
	H	305 ±	4.0	280 ±	2.6	585	0.91

Discussion

Our results corroborate the assumption that in humate-treated plants especially in roots, the role of glycolysis in total respiration is increased. This is supported by the increased action of the respiratory inhibitors used as well as by the higher stimulation and the increased ratio C₆/C₁ in roots. The degree

Tab. 5. Production of $C^{14}O_2$ from 3-day-old roots of wheat grown in water and in a solution of Na-humate 100 mg./l. after four-hour incubation with unspecifically labelled glucose

Experiment	Radioactivity of $BaC^{14}O_2$ in counts/min./10 mg. dry weight	
	Na-humate	distilled water
	$\bar{x} \pm s\bar{x}$	$\bar{x} \pm s\bar{x}$
17. 10. 61	227.0 \pm 4.05	253.0 \pm 6.45
12. 12. 61	274.5 \pm 28.4	461.8 \pm 12.8
19. 12. 61	250.0 \pm 23.6	331.0 \pm 9.54

of inhibition of respiration by fluoride and monoiodoacetate is proportional to the part of glycolysis in glucose catabolism. Malonate does not affect the glycolysis directly, but it can inhibit it unlike the pentose cycle because glycolysis is dependent on the citric acid cycle in aerobic conditions in tissues of higher plants. The increased respiration inhibition by malonate in roots treated by humic acid can thus be an indirect corroboration of the above assumption. Though ŠMÍDOVÁ (1962) found a decreased inhibitory action of malonate on the consumption of oxygen in the root homogenate of humate-treated plants, this result does not contradict ours, because there is a considerable difference between the homogenate and an intact tissue. The stimulation of respiration in wheat leaves by fluoride is accompanied by an increased C_6/C_1 ratio and inhibition of the transport of glucose into the tissues, which is probably a result of activation of glycolysis (LUŠTINEC, POKORNÁ, RŮŽIČKA 1962). It is evident that under favourable conditions the respiration will be most strongly stimulated by fluoride when the role of glycolysis in tissue respiration is at the highest level. But it may happen that during the transition of the inhibitory effect to stimulation, caused by a changed concentration of the enzyme-poison or by the age of the tissue, respiration including intense glycolysis will be inhibited or stimulated less than respiration of the tissue where aerobic glycolysis is partly supplied by the pentose cycle (Fig. 1). Our opinion is that analogical relations may be expected for the action of monoiodoacetate and malonate. According to the results of manometric experiments with inhibitors increased ratio C_6/C_1 in the roots of the humate-treated plants may be caused by increased glycolysis. KATZ and WOOD (1960) showed that the C_6/C_1 ratio is not a linear function of a relationship of glycolysis and the pentose cycle in catabolism, but that it also depends on an amount of 3-C from triosophosphate, oxidized in CO_2 and on the non-triosophosphate utilisation of glucose. It is thus possible in wheat leaves cultivated in humate solution whose respiration is more strongly inhibited by fluoride than that of the control, that the C_6/C_1 ratio corresponds to the higher role of glycolysis as well.

The found metabolic changes are correlated with an intense growth of leaves and especially of roots, but it is impossible to say from our experiments how specific the influence of humic acid is. The experiments on the short-term influence of humate on isolated organs of wheat showed that during several hours application no metabolic changes occur; this might be caused by the slow transport of humic acid into tissues (PRÁT 1960).

Whether the decreased $C^{14}O_2$ radioactivity in roots is caused by the decreased transport of glucose to the tissues or by other factors cannot be said at present.

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Влияние Na-гумата на дыхание корней и листьев пшеницы

Фторид натрия, монойодацетат и малонат тормозят, в других случаях стимулируют дыхание корней пшеницы, выращиваемой в течение 2—10 дней в растворе гумата натрия (100 мг/л) сильнее чем дыхание корней растений выращиваемых в воде. Подобным образом действует фторид натрия на дыхание листьев. Отношение радиоактивностей $C^{14}O_2$ освобожденного из глюкозы меченой в 1-ом или 6-ом положении (C_6/C_1) достоверно повышено у корней, однако, не у листьев. Изменения этого отношения сопровождаются уменьшением общей радиоактивности $C^{14}O_2$ освобожденного корнями растений, на которые воздействовали гуматом, из глюкозы специфически и тотально меченой. Эндогенная респирация (QO_2) корней под действием гумата повышается на 5—30%, интенсивность респирации листьев остается на том же уровне. Рост корней в длину в среде с гуматом на 20—80% интенсивнее, рост листьев на 5—15%. Приведенные данные ведут к заключению, что в корнях растений, выращиваемых в растворе гумата, возрастает доля гликолиза в респирационном метаболизме.