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INFLUENCE OF DEUTERIUM CONTENT OF WATER ON THE GROWTH OF PLANT EMBRYO AND FREE RADICAL PRODUCING PROCESSES

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Abstract

In the course of germinating plant seeds - if the deuterium (D) concentration in the germinating medium (water) is lower or higher than in natural water (150 ppm) – the length and mass of seedlings (coleoptil, epi-cotyl, root) proportionally decrease. The cause of the decrease owing to the changed D concentration is explained partly on energetic-partly on free radical basis and proved by measuring. In waters with lower or higher than na-tural D concentration the energy supply decreases while the free radical formation in-creases. Free radicals exert their influence by inactivating the bios substances (e.g. auxin) through oxidation. This seems to explain the mechanism of the ensuing changes.

INTRODUCTION

Earlier we gave an account on the effect exercised by the medium of changed deuterium content (D20, heavy water) on the division of cells and growth of seedlings (Kiss et al. 1996, 1997, 1998, Kiss 2003).

Deuterium is a nonradiating mass number 2 isotope of hydrogen. The 100% mass difference appears in chemical reactions and biological effects of the two hydrogen atoms (H and D), e.g. *Aspergillus niger* raised in "light water" is black, while moulds grown in heavy water are alabaster white.

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The rate of reaction of deuterated compounds is lower, since compounds of greater mass have a higher requirement of activation energy. According to the investigations of SIMMON and PALM (1966) the activation energy of the deuterated succinate dehydrogenase is 13% higher compared to the enzyme which contains natural hydrogen. Plants grown in culture media of increasing D content are consequently depressed in growth e.g. germination of maize and growth of coleoptiles (SACCHI and COUECHI, 1992).

SOMLYAI et al. (1993) found that under the influence of a decreasing D concentration the division of cells in various animal tissue cultures started with a 5–10 hour delay. The inhibitory effect can be experienced in tumorous animal tissues too. This was the first case when a D content lower than the natural deuterium concentration (150 ppm) was found to inhibit the propagation of cells.

Subsequently we also began to study the influence of a slight (50–130 ppm) change in the D content of a culture solution on the development of plants, using various seeds for that purpose. Our work differed essentially from earlier investigations (UPHANS et al 1975), where the effect of 30–70% D20 contents on cell propagation was studied, as we kept close to the natural (150 ppm) concentration.

We observed that when the D content in a germinating medium was either lower of higher than the deuterium concentration in natural waters, the growth of mono- and dicotyledonous seedlings was inhibited in proportion to the difference (Fig. l., KISS et al. 1998).

The present paper gives an account on the causes and possible mechanism of the inhibitory effect.

MATERIALS AND METHOD

In our germination experiments we used distilled water of 20-300 ppm D content that we produced by mixing "light water" (20 ppm) with heavy water of 99.689% D content at an appropriate ratio, and storing it in dark glass containers. The contamination was controlled by atomic absorption spectrophotometry.

GERMINATION

Maize and pea seeds of 95% germinability were germinated in high covered plastic Petri dishes on filter paper wetted with identical quantities of water. Each treatment consisted of 5 replications, with 20 seeds per dish.

DETERMINATIONS

We measured the length and mass of epicotyl and coleoptile as well as the mass of root during germination (after 6 days). To determine the organic matter (energy) utilization and the respiratory loss we measured dry weight of maize coleoptiles and roots, and the decrease in dry matter content of seed during germination. The samples were dried at 95°C to standard weight, and then measured.

The total activity of the "bios material" was determined by yeast test method. (Bios material = growth stimulating hormones such as auxin, gibberellin, etc). The bios materials exercise activity stimulated the propagation of yeast cells → the decomposition of sugar in the culture medium. The CO_2 produced leaves the culture medium resulting in a decrease of mass.

Fixed volumes of the supernatant obtained after centrifuging maize coleoptile homogenate were added to the yeast culture. The release of CO_2 was possible through a plug (0.1 mm) closing the vessels. We kept the cultures in a thermostat at 30°C and recorded every day the total weight of vessels and cultures; from the loss of weight we assessed the activity of the bios material.

To characterize free radicals of oxygen we determined the lipid peroxidation (LP), the hydroxyl radical ($OH\cdot$), the superoxide dismutase (SOD), catalase (Cat) enzyme activities as well as the total antioxidant capacity (FRAP).

PREPARATION OF THE SAMPLE

A predetermined quantity of shoots was homogenized in a Potter homogenizer in cold phosphate buffer of 1:9 (w/w) and 7.4 pH. Aliquots of the supernatant obtained after centrifuging (2000 rpm) were used to determine various parameters.

The superoxide dismutase [(SOD, EC 1.15.1.1) activity was determined by the method of Misra and Fridovich (1972) modified by Matkovics et al. (1977).

The rate of lipid peroxidation (LP) was measured by thiobarbituric acid colour reaction after Placer (Placer et al. 1966).

For the determination of the hydroxyl radical ($OH\cdot$) we employed Cheeseman's method (Cheeseman et al., 1988).

Catalase (Cat: EC 1.11.1.6) activity was measured after Beers and Sizer (1952), and the activity was given in Bergmeyer unit (BU), where 1 BU = 1000 mg H_2O_2 decomposed in 1 minute.

The protein concentration was determined as described by Lowry et al. (1951).

The total antioxidant activity was determined by the method of BENZIE and STRAIN (1996).

RESULTS

When germinating maize, rice, gourd and wheat seeds in media of 25, 150, 300 ppm deuterium concentration we found that the length and mass of shoots (coleoptile, epicotyl) were the largest at a D concentration of 150 ppm, i.e. corresponding to that in natural waters. Lower and higher deuterium concentrations had an inhibitory effect on growth (Fig. l.)

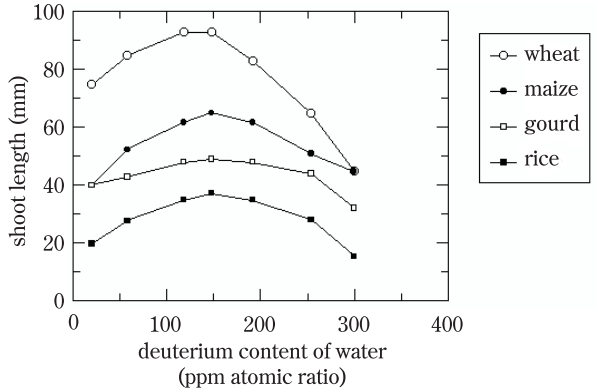

Fig. 1. Shoot length of seedlings as a function of the D content of water (KISS 1998) (the data represent mean values of several species of wheat, maize, gourd and rice seedlings)

The decrease in the mass of seeds during germination is the largest in the medium containing 150 rpm D (Table 1), while in the case of 25 and 300 ppm it is lower. The differences for the three maize hybrids, though considerable, were consistent.

The increase in the total shoot + root mass of seedlings in the first 10 days is, according to WILLIAMS-PETERSON (1973), proportional to the α-amilase activity of seeds, that is to the composition of yeast. According to our investigations, the increase in the mass and α-amilase activity was the highest in the medium of 150 rpm concentration. This is in harmony with a decrease in the mass of germinated seeds. In the course of germinating, comparing the decrease in the mass of seeds with that in the total dry mass of shoots and roots we found the latter to be lower in every case (Table 2). The difference is the so called respiratory loss, which has been spent on the

Table 1

Mass decrease of seeds of *Zea mays* L. varieties during germination as a function of the deuterium con-centration in a medium (water). The data are given as percentages of the original mass and in comparison to 150 ppm, means from 5x20 seeds

Species/Mass decrease (%)	Concentration of deuterium (ppm)		
	25	150	300
Ida			
absolute decrease, %	10.5	11.6	10.0
decrease compared to D-150, %	90.5	100.0	86.2
Raissa			
absolute decrease, %	22.4	24.1	22.1
decrease compared to D-150, %	92.9	100.0	91.7
Randa			
absolute decrease, %	19.8	21.4	19.6
decrease compared to D-15.0, %	92.5	100.0	91.5
Mean decrease, %	17.6	19.0	17.2

Table 2

Correlation between the coleoptile + root mass of *Zea mays* L. var. Ida germinated in media of various deuterium contents and the weight loss of seeds (mg/weight of 20 seeds), as percentage value of the respiratory (energy) loss, average of three varieties and in comparison to 150 ppm D content

Quantity (mg) and loss of quantity	Concentration of deuterium (ppm)		
	25	150	300
Coleoptil	184	210	182
%/D-150	88.0	100.0	87.1
Root	604	706	640
%/D-150	85.4	100.0	90.6
Coleoptil+root	788	916	822
%/D-150	86.0	100.0	90.6
Ratio of coleoptil/root	3.25	3.26	3.50
Mass decrease of seeds	1206	1350	1156
Respiratory loss, mg	414	434	382
Respiratory loss, %	34.3	32.1	33.0
%/D-150	106.8	100.0	102.8

Respiratory loss = mass decrease of seed - (coleoptile + root) mass

energy transformation. The lowest respiratory loss was measured at a 150 ppm D concentration. From this we have drawn the conclusion that the energy supply (transformation) varies with the deuterium concentration.

The assumption that owing to the changed D concentration the energy supply decreases in the course of germination seems to be supported by the fact that ATP (3 mg 100 ml^{-1}) added to the germinating medium influences the growth of the epicotyl as a function of the D content (Table 3). The growth of the epicotyl was proportionally higher in a medium of either decreased (25 ppm) or increased (300 ppm) D concentration than in the case of a natural (150 ppm) level of D content. This proves that with a deviation from 150 rpm the energy (ATP) production decreases and so does the transfer entropy (HASTEN et al. 1974), and its substitution resulted in a spectacular growth.

Table 3

Changes in the shoot (epicotyl) length, oxygen free radical- and antioxidant activity of germinating pea (*Pisum sativum* var. Hanka) as a function of the deuterium and ATP content of a medium (average of 5 replications)

Parameters	Deuterium (ppm)						Deuterium (ppm)+ ATP (mg ml^{-1})					
	28		150		300		28 + 30		150 + 30		300 + 30	
		%		%		%		%		%		%
Epicotyl, mm	12.3	67	18.3	100	14.0	76	19	+54	24.0	+31	22.1	+58
LP, nM mg^{-1}	18.1	111	16.2	100	17.9	110	18.2	0	17.2	+10	19.2	+7
$\cdot$OH nM mg^{-1}	23.2	123	18.9	100	21.2	112	22.1	-5	20.4	+8	21.8	+3
SOD, E mg^{-1}	14.8	98	15.1	100	15.0	99	15.2	+2	14.9	-2	16.0	+6
Cat, E $mg \cdot 10^{-4}$	0.8	108	0.7	100	0.7	100	1.28	+66	1.30	+83	1.20	+74

Effects of ATP in % = in proportion to the ATP-free, deuterated solution

The determination of the so called bios substances (auxin, gibberellin, etc.) in maize coleoptiles shows that their activity is a function of the deuterium concentration in a germinating medium (Table 4). The data are fully consistent with the growth of coleoptiles and the change of the α-amilase activity.

Values for the concentration of oxygen free radicals and the activity of antioxidants eliminating them are seen in Table 5. Lower hydroxyl radical and lipid peroxidation (LP) values as well as higher antioxidant enzyme (SOD, Cat) and total antioxidant (FRAP) activities are obtained in the case of a concentration corresponding to the deuterium concentration of natural waters.

Table 4

Comparison of *Zea mays* L. var. Ida coleoptiles grown in media of different deuterium concentrations for bios activity, on the basis of mass decrease in sugar-yeast solution, versus the mass decrease of seed during germination and the mass of coleoptil (mg n^{-1})

Parameters	Concentration of deuterium (ppm)		
	25	150	300
Quantity of colepotil, mg n^{-1}	56.58	126.53	77.36
%/D-150	44.7	100.0	61.1
Mass decrease, mg n^{-1}	42.48	46.50	41.04
%/D-150	89.5	100.0	88.2
Mass decrease of sugar-solution mg n^{-1} coleoptil			
During 12 hrs	1.72	2.00	1.55
+ 13 hrs	3.28	4.11	4.04
Total: during 25 hrs	5.00	6.11	5.59
%/D-150	81.8	100.0	90.7

5 parallel, mean for 20-20 seeds

Table 5

Oxygen free radical and antioxidant enzyme ac-tivities of coleoptiles of maize (var. lda) germinated in media of various deuterium concentrations

Parameters	Concentration of deuterium (ppm)		
	25	150	300
Protein, mg g^{-1}	22.70	23.25	22.50
%/D-150	97.6	100.0	96.7
LP, nM MDA mg^{-1} prot.	4.06	3.75	4.15
%/D-150	108.3	100.0	110.6
·OH, nM mg^{-1} prot.	43.6	34.5	39.3
%/D-150	126.4	100.0	113.9
Cat, E mg^{-1} prot. $\cdot 10^{-4}$	1.059	0.972	0.969
%/D-150	108.9	100.0	99.7
SOD, E mg^{-1} prot.	14.3	15.6	13.4
%/D-150	91.6	100.0	85.9
FRAP values, µM L^{-1}	252	276	239
%/D-150	91.3	100.0	86.6

DISCUSSION

According to SIMMONDS and DUMBROFF (1974) germination of seeds starts only at an ATP level (EC = energy charge) characteristic of a given species. In our experiments, the EC level in seeds was raised by an ATP treatment, germination started earlier and was more intensive than without such a treatment (KISS 1983). With D concentrations lower or higher than 150 ppm the effect of ATP was essentially more profound than in the case of 150 ppm.

HAILSTONES and SMITH (1988) pointed out that while the number of oxygen free radicals increase, the germinabi1ity of seeds decreased, e.g. in soya bean a 40% increase in the H_2O_2 inhibited germination. LEE (1972) attributed this phenomenon to a decreasing effect of peroxidation on the auxin level and consequently on growth. Similar conclusions were drawn by GASPAR et al. (1985): increasing peroxidation oxidizes auxin whereby it becomes inactive.

Increasing lipid peroxidation (LP), which is related to the damaging effect of oxygen radicals, is due to the fact that in a medium of low D content the SOD activity decreases, and so does the possibility of eliminating radicals (AUROMA et al. 1989). According to our investigations, with ATP added the quantity of oxygen radicals did not increase, neither did that of LP. The oxidative circumstances did not diminish the auxin level, therefore the growth of seedlings was intensive.

In our experiments the concentration of the hydroxyl radical and the rate of LP increased in a medium lower in D. The cause is that a lower D_20 concentration increases the disorder of the structures. Increased disorder means increased entropy (S) too, which tends to make the reactions spontaneous. Thus, the hydroxyl radical formation and the process of LP take place more easily and rapidly. A similar result was attained by HANSTEN et al. (1974), who found oxidation of the NADH-Coenzyme Q increased with a decrease in the D content of a medium.

Besides, with a decrease of the D concentration, the ATP-ase activity in mitochondria and NADH formation increase (GOMEZ-PUJOU et al. 1978). The increasing NADH concentration promotes formation of free radicals (PUNTARULO et al. 1988), which results in an increase of LP.

Higher production of free radicals in a medium of lower D content raises the activity of Cat, which takes part in their elimination. Supplementary ATP addition contributes by 60–80% higher Cat activity, probably by increasing the de novo synthesis. With increasing deuterium concentration oxygen uptake by plants decreases, and so does the ATP formation (SACCHI, COUECHI 1992). As a joint effect of energy decrease and free radical formation increase, the growth of seedlings under higher D content in a medium is inhibited. D_2O has a selective inhibitory effect on the mitochondrial energy transfer (MARGOLIS et al. 1966).

CONCLUSION

Germination of plant seeds and growth of shoots and roots depend on the deuterium content in a germinating medium. If the D content of a medium is lower or higher than the 150 ppm D concentration in natural waters, the growth or seedlings will be inhibited.

The growth inhibition or seedlings is partly caused by a decrease in energy or its transfer, in part due to the inactivation or auxin through a raised level or oxygen free radicals. The latter conclusion is supported by our "bios material" measurments.

If the energy level is raised by adding ATP, the growth inhibition caused by a change in the D concentration will cease. We can declare that the division of cells and the growth of plants is optimum when the deuterium content is around 150 ppm, a level developed in the course of evolution.

REFERENCES

AUROMA OI., HALLIWELL B., GAJEWSKI E., DIZDAROGLU M. 1989. *Damage to the bases in DNA induced by H_2O_2 and ferric ion chelates.* J. Biol. Chem., 264: 20509-20512.

BEERS RF., SIZER IW. 1952. *Spectrophotometric method for measuring the breakdown of hydrogen peroxyde by catalase.* J. Biol. Chem., 195: 133-140.

BENZIE IIF., STRAIN JJ. 1996. *The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay.* Anal. Biochem., 239: 70-76.

GASPAR T., PANEL C., CASTILLO FJ., GREPPIN H. 1985. *A two-step control of basic and acidic peroxidases and significance for growth and development.* Physiol. Plant., 64: 418-423.

GOMEZ-PUJOU TT., GOMEZ-PUJOU A., CERBON J. 1978. *Increased conformational stability of mitochondrial soluble ATP-ase (F1) by substitution of H_2O for D_2O.* Archiv. Biochem. Biophys. 187: 72-77.

HAILSTONES MD., SMITH MT. 1988. *Lipd peroxidation in relation to declining vigour in seeds of soya and cabbage.* J. Plant Physiol., 133: 452-456.

HANSTEN W.G., DAVIS K.A., HATEFI Y. 1974. *Solvent isotope effects on the structure and function of mitochondrial membranes in aqueous media*. Archiv. Biochem. Biophys., 163: 482-490.

KISS AS. 2003. *Deutérium az új mikroelem és annak biokémiai hatásai.* Magyar Kémikusok Lapja, 58: 41-45 (in Hungarian).

KISS AS. 1983. *Magnéziumtrágyázás, magnézium a biológiában.* Mezogazdasági Kiadó, Budapest, 110-115 (in Hungarian).

KISS AS., GALBÁCS Z., GALBÁCS G. 1997. *Magnesium ions hinder the growth on coleoptyl in deuterium depleted water.* In: *Current status and new developments.* THEOPHANIDES T., ANASTASSOPOULOU J. Eds. Kluver Acad. Publ., 77-80 pp.

KISS AS., GALBÁCS Z., KAMASZ-SÁRI V., GALBÁCS G. 1996. *Impact of the deuterium concentration of water and magnesium salts on the germination seeds.* Magnes. Res., 9: 226-227.

LEE TT. 1972. *Interaction of cytokinin, auxin, and gibberellin on peroxydase isoenzymes in tobacco tissue cultured in vitro.* Canad. J. Botany, 50: 2471-2477.

LOWRY OH., ROSEBROUGH NJ,. FARR AC., RANDALL RJ. 1951. *Protein measurement with Folin phenol reagent.* J Biol. Chem., 193: 265-275.

MARGOLIS SA., BAUM H., LENAZ G. 1966. *Observations on the effect of D2O on energy linked reactions of the mitochondrion.* Biochem. Biophys. Res. Commun., 25: 133-141.

MATKOVICS B., NOVÁK R., HOANG DUC HAHN, SZABÓ L., VARGA SI. 1977. *A comparative study of some more important experimenta animal peroxide metabolism enzymes.* Comp. Biochem. Physiol., 56B: 31-37.

MISRA HP., FRIDOVICH I. 1972. *The role of superoxide anion in the autoxidaton of Epinephrine and simple assay for superoxide dismutase.* J Biol. Chem., 247: 3170-3176.

PLACER ZA., CUSHMANM L., JOHNSON BC. 1966. *Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical system.* Anal. Biochem., 16: 359-364.

PUNTARULO S., SANCHEZ RA., BOVERS A. 1988. *H_2O_2 metabolism in soybean embryonic axes at the onset of germination.* Plant Physiol., 86: 626-630.

SACCHI GA., COUECHI M. 1992. *Effect of D_2O on growth, proton extrusion, potassium influx and in vitro plasma membrane activities in maize root segments.* Plant Physiol., 100: 192-196.

SIMMON H., PALM D. 1966. *Isotope effects in organic chemistry and biochemistry.* Angewandte Chemie (Int. ed. in English), 5: 920-933.

SIMMONDS JA., DUMBROFF EB. 1974. *High energy charge as requirement for axis elongation in response to GA and kinetin during stratification of Acer saccharum seeds.* Plant Physiol., 53: 91-95.

SOMLYAI G., JANCSÓ G., JÁKLI G., VASS K., BARNA B., LAKICS V, GAÁL T. 1993. *Natural occuring deuterium is essential for normal growth rate of cells.* FEBS Lett., 317: 1-4.

SZALAY I., FRENYÓ V. 1962. *Növényélettani Kisérletek.* Tankönyvkiadó Budapest, 356-357 (in Hungarian).

UPHANS RA., BLAKE MI., KATZ JJ. 1975. *Deutrium isotope effects in Nicotiana tabacum.* Canad. J. Botany, 53: 2128-2131.

WILLIAMS JF., PETERSON ML.. 1973. *Relations between alfa-amylase activity and growth of rice seedlings.* Crop Sci., 51: 612-615.