Volume 47(1-4):127-130, 2003
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

SYMPOSIUM

Effect of age and magnesium supply on the free radical and anti-oxidant content of plants+

Sándor A. Kiss[1], Ilona Szöllősi Varga[2]*, Zoltán Galbács[3], Takács-Hájos Mária[4], Csikkel-Szolnoki Anna[3]

[1]Hungarian Magnesium Society, [2]Department of Genetic and Molecular Biology, University of Szeged, Szeged, Hungary, [3]Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged, Hungary, [4]College Tessedik Sámuel, Department of Agriculture, Water and Environmental Management, Szarvas, Hungary

ABSTRACT The scientific literature signs that the shortage of magnesium may increase the free radical content in the animal and human tissues, but nothing can be kown about a similar effect in the plants. This is why we decided to research the plant tissues to know how the radical content and the concentration of antioxidants which may eliminate the free radicals. We have researched the wheat (*Triticum aestivum* var. GK Pinka), maize (*Zea mays* var. Furio) and table beet (*Beta vulgaris* var. Rubra). We measured the concentrations in the coleoptyle of wheat and maize and in the roots of table beet. On the basis of our measurements we may conclude that the increase of magnesium concentration in the nutrient solution does decrease the radical content ($HO^{\bullet}$, LPO) and the activity of antioxidants. It is known, that the production of free radicals does increase as the uncoupling of oxidative phosphorilation is increasing according to its measure. Nevertheless the reduction of concentration of magnesium increases the measure of uncoupling of oxidative phosphorilation and the production of free radicals. On the basis of these effects one can understand why the radical content is lower after the addition magnesium ions into the nutrient solution. The decrease of activity of activity of GSH, catalase and FRAP-value may be understood also: the real cause is the fact that the promoter free radicals needed to the "de novo synthesis" have decreased. The last conclusion may be the next: the magnesium does influence the of free radicals and antioxidants in the plant tissues.

KEY WORDS

free radicals
anti-oxidant content
magnesium supply
wheat
maize
table beet

Acta Biol Szeged 47(1-4):127-130 (2003)

Scientific papers declared that the shortage of magnesium in the animal cell and tissue does increase the free radical content of cell. Kramer et al. (1994) reported that radical content in the heart tissue supplied not enough magnesium was two times higher as in the control tissue supplied by enough magnesium. Wiles et al. (1997) demonstrated that the concentration of free radical containing oxygen did decrease (with 10-15%) when the concentration of magnesium decreased in the aortic endothelial cell. Stafford et al. (1993) presented, that the hydroperoxide level in the rat blood increased in case of magnesium shortage.

We could not find similar phenomena and date in plant cell and tissue. That is why we wanted to know the effect of magnesium on radicals and antioxidants.

Materials and Methods

We have researched the seeds of wheat (*Triticum aestivum*, var. GK Pinka) and maize (*Zea mays*, var. Furio) and the root of table (red) beet (*Beta vulgaris*, var. Rubra).

Accepted April 30, 2003
*Corresponding author. E-mail: szvarga@biocom.bio.u-szeged.hu
+In memory of Professor Béla Matkovics

The germination was carried out in a plastic (PE) dish (which was similar to the Petri dish). The grains were placed on a filter paper covering cotton-wool soaked in distilled water or in solution of magnesium sulphate (0.1% and 1% m/v $MgSO_4 \bullet 7H_2O$). The temperature was 22-24ºC during the germinating period. On the 6-8-12th days the shoot (of approximately 25 mm long) was separated. The weighted portion of shoot was homogenised with a Potter type homogeniser and during the process the effective cooling did not allow the temperature increase. The solution used for homogenisation was made of phosphate puffer solution and EDTA (pH = 7,6; EDTA = 1 mM) and it was adjusted to the composition of 1 part shoot to 4 part solution (1:4). After the homogenisation the mixture was centrifuged (5 min 10,000 g) and the upper layer was used for measurements.

We determined the free radical contents in shoots (coleoptyl, epicottyl) of different ages as well as the upper and lower half of coleoptyl. In the case of the coleoptyl, the upper half is younger than the lower half, and we wanted to test whether this slight difference in developmental age would case any detectable difference in the concentration of free radicals.

Table 1. Effect of magnesium on germination of wheat.

Measured components in the coleoptyle of wheat	Measured value Control Without Mg	With 0,1% $MgSO_4 \bullet 7H_2O$	Difference compared	Measured value with 1% to control %	Difference compared $MgSO_4 \bullet 7H_2O$ to control %
Protein, mg/g	11,66	12,12	+4	11,96	+3
LPO, nM MDA/mg prot.	6,7	5,5	-18	5,7	-15
$OH^{\bullet}$, nM MDA/mg prot.	47,4	43,1	-9	41,9	-12
FRAP value, (mM Fe(II)/L)	532	472	-11	372	-30

Table 2. Effect of magnesium on germination of maize.

Measured component in the coleoptyle of maize	Measured value in the control	Measured value at treating with 1% $MgSO_4 \bullet 7H_2O$	Difference compared to the control %
Protein, mg/g	23,25	24,79	+6,6
LPO, nM MDA/mg prot.	3,75	2,08	-45,0
OH^{Σ}, nM MDA/mg prot.	34,50	18,50	-46,0
GSH, mM/mg prot./10^{-2}	5,67	2,55	-55,0
Catalase, E/mg prot./10^{-4}	0,972	0,316	-67,0
FRAP value, (μM Fe(II)/L)	266	200	-25,0

We also investigated the effects of the total mass and total protein content.

The root of table beet was prepared similar to the kitchen process. After cleaning and the separation the outer layer it was grated with a fine kitchen grater. A weighted portion after addition of distilled water to be adjusted the 1:9 mixture was homogenised with the Potter type homogeniser. As the mixtures made of table beet were very violet-coloured we could measure only the magnesium content and the FRAP value.

We used a magnesium solution (1% m/v $MgSO_4 \bullet 7H_2O$) for spraying the leaves of red beet at growing to research the effect of magnesium on the growing. The spaying was repeated two times per day during the researching period (2 weeks) and the quantity was 0.2 l/m^2 leaves.

The protein content was measured according to Lowry et al. (1951).

The hydroxyl free radicals were determined according to the method of Halliwell and Gutteridge. The $HO^{\bullet}$ radicals at low pH react with 2-deoxy-D-ribose and thiobarbituric acid giving a red coloration. Colour intensity was measured at 532 nm.

LPO activity was measured according to Plancer et al. (1966). The determination is based on the LPO-catalysed reaction of malone-dialdehyde (MDA) with thiobarbituric acid (TBA). The product of reaction is coloured and the colour intensity was measured at 532 nm.

It was determined according to Tietze (1969). The reaction of GSH with the Elman-reagent DTNB (5,5-dithiobis-[2-nitro-] benzoic acid) yielded a yellow coloration measured at 412 nm.

The determination was made at 240 nm according to Beers et al (1952). The concentration of hydrogen peroxide was controlled in 0,05 M phosphate puffer at pH, measuring the absorbency of solution at 240 nm in silica cell. The enzyme activity was given in Bergmeyer (Be) units (decomposition of 1 g H_2O_2/min at 25ºC).

The effect of antioxidant was determined according to

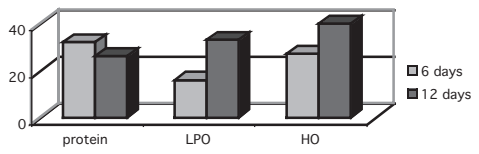


Figure 1. Protein, LPO and $HO^{\bullet}$ content in the 6 and 12 days coleoptyl of maize.

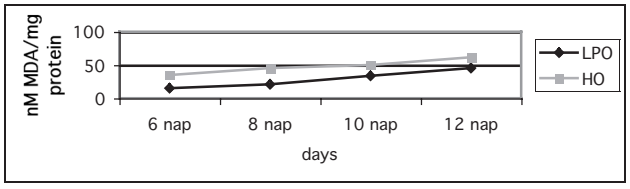


Figure 2. Change of the content of LPO and $HO^{\bullet}$ in the coleoptyl of maize depends on its developmental age.

Table 3. The magnesium content and FRAP value as a function of the species of red beet.

Species of red beet	Mg content, mg/kg			FRAP value		
	Control	Spraying with 1% $MgSO_4 \cdot 7H_2O$	Difference compared to control %	Control	Spraying with 1% $MgSO_4 \cdot 7H_2O$	Difference compared to control
Bordó	1970	2030	+2	330	300	-9
Detroit	2410	2600	+8	312	184	-41
Favorit	1970	2030	+3	343	317	-8
Nero	2040	2160	+3	344	246	-28
Rubin	2130	2270	+7	337	177	-47

Benzie-Strain (1996). The method based on effect of antioxidants to be able to reduce the Fe(III) to Fe(II) in a complex compound (Fe(III)-tripyridine-triazine). The product Fe(II)-compound is blue-coloured and its intensity is measured at 593 nm. After measuring the absorbancy of the blue-coloured solution the concentration can be determined using the calibration curve made by known Fe(II) solutions.

The samples were digested by concentrated HNO_3 solution in microwave oven (0.2 g sample + 5 ml cc HNO_3). After the digestion the solution was filtered and diluted to 50 mL with distilled water. The concentration measurement were made on Jobin Yvon 24 ICP AES instrument.

The spectrophotometric measurements were conducted on the SPECTROMOM 360 and 202 type instruments.

Results

Wheat

The addition of magnesium ion into the germination solution decreased the concentration of hydroxyl free radicals ($HO^{\bullet}$), and the value of lipid peroxidation (LPO) and FRAP value. In all case the addition of magnesium increased the amount of protein (Table 1).

Maize

All the measured parameters (LPO, $HO^{\bullet}$, GSH, catalase activity, FRAP value) decreased after the addition of magnesium. (Table 2; Fig. 1). LPO and $HO^{\bullet}$ increased with time (Fig. 2).

Red beet

The fertiliser magnesium (spraying on the leaves) increased the concentration of magnesium and decreased the FRAP value. The effect of magnesium does differ according to the species, but the tendency is similar. (Table 3).

Figure 3 shows the differences of protein, LPO and HO contents between the upper and lower halves as well as the whole coleoptyl. As we can see, the relatively small age difference within the shoot causes a significant deviation in these values, so that one has to take into consideration this fact in collecting shoot samples.

Figure 4 represents the changes of whole protein, LPO, HO, catalase and SOD values in the younger and older epicotyl halves and whole epicotyl of pea plants.

Figure 5 shows the difference when the values are standardised to the usual whole protein content or the total mass.

Discussion

On the basis of our measurements we may conclude that the increase of magnesium concentration in the nutrient solution does decrease the radical content ($HO^{\bullet}$, LPO) and the activity of antioxidants. It is known, that the production of free radicals does increase as the uncoupling of oxidative phosphorilation is increasing according to its measure. Nevertheless the reduction of concentration of magnesium increases the measure of uncoupling of oxidative phospho-rilation (Vitale et al. 1957) and the production of free radicals. On the basis of these effects one can understand why the radical content is lower after the addition magnesium ions into the nutrient solution.

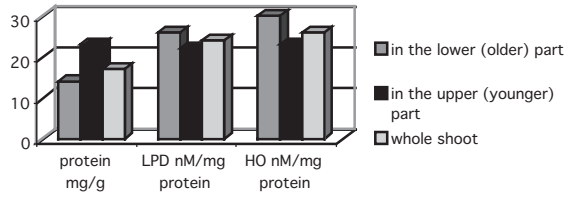
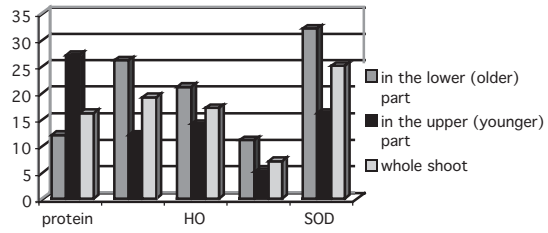


Figure 3. Protein, LPO and $HO^{\bullet}$ content in the 10 days coleoptyl of maize.



Fiure 4. Protein, LPO, $HO^{\bullet}$, catalase and SOD content in the coleoptyl (epicotyl) of pea.

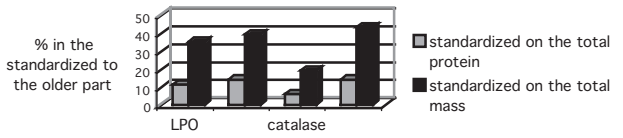


Figure 5. Change of the % of LPO, HO•, catalase and SOD values depend on the age of coleoptyl, standardized to the whole protein content or total mass.

As we can see, the activity of the oxygen free radicals and the anti-oxidants depend on both the age of the whole shoot (coleoptyl and epicotyl) and the slight difference in developmental age between the different parts of the same shoot. The basis of standardisation (protein content or total mass) can cause great differences in the free radical and anti-oxidant values. If standardised to the protein content, the free radical and anti-oxidant values are lower in younger specimens and grow with age. It can be explained with the protein content being higher at younger age than at the older one. When the values are standardised with respect to the total mass, the correlation with age in the opposite. In our opinion, standardisation to the total mass can better reflect reality in certain cases.

The decrease of activity of GSH, catalase and FRAP-value may be understood also: the real cause is the fact that the promoter free radicals needed to the "de novo synthesis" (Vanacker 1998) have decreased.

The last conclusion may be the next: the magnesium does influence the activity of free radicals and antioxidants in the plant tissues, as it was described in case of animal tissue and cell.

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