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ELECTRON PARAMAGNETIC RESONANCE (EPR) INVESTIGATIONS OF LASER INDUCED FREE RADICALS IN SPRING WHEAT GRAINS*

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A b s t r a c t. Electron paramagnetic resonance (EPR) spectroscopy was applied to the studies on free radicals in wheat grains. The stable free radicals normally present in cereal grains can be detected by EPR measurements. Concentrations of the radicals in investigated grains were in the order of 10^{16} spins/gram of dry mass. The single signal characteristic for oxygen-containing (semiquinone) radicals at g=2.0047 was observed as a dominating EPR signal; a small admixture of an additional radical at g=2.006 was also detected. The He-Ne and semiconductor laser irradiadion of the grains (doses 10^{-5} to 10^{-1} J/cm^2) resulted in a distinct increase of the stable radical concentration (signal at g=2.0046). The increase of 10 to 30% depending on the laser irradiation technique was measured; the dependence on the dose and the cultivar was less significant. The new radicals have transient character and EPR signal of the laser irradiated grains gradually decreases for several days to stabilize at the normal level of the radical concentration.

K e y w o r d s: wheat grain, laser, electron paramagnetic resonance (EPR), g-factor, free radical

INTRODUCTION

The free radical reactions are characteristic for all living organisms. The level of free radicals in living organisms is precisely controlled by special enzymes and chemical systems generating or scavenging the radicals. The physical parameters (temperature, irradiation, redox potential) also influence the free radical concentration. The reactive radicals in living organisms are usually short-lived and can not be detected directly using normal EPR technique; however, the spin-trapping technique makes detection of the radicals possible.

The natural polymers (phenolic polymers, melanins, melanoidins, etc.) can act as spin traps in various biological systems; we detect the radicals stabilized in the polymeric matrices.

Stable free radicals were found in various food products containing starch and other polysaccharides by Yordanov and Stoilova-Ivanova [11]; in the heated protein-carbohydrate mixtures, e.g., wheat protein-starch system by Friedman [3]; living and degraded plants by Lisowski, Jezierski and Bylińska [9]; in microorganisms by Golab, Breitenbach and Jezierski [4]; in organic matter in soil and composts by Jezierski *et al.*[6]; in archeological cereal grains by Hillman *et al.*[5].

In general, the irradiation of polyphenolic or melanoidin polymers (e.g., Maillard reaction products) results in the enhancement of semiquinone radical concentration according to Senesi [10]. In the cereal grains the partially

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degraded polysaccharides and the Maillard condensation products can act as natural matrices stabilizing free radicals. In this work we present the quantitative EPR investigations on stable radical concentration in laser-irradiated wheat grains. Laser radiation is used as an effective biostimulating method for wheat grains in the form of pre-sowing irradiation of seeds according to Drozd [2].

MATERIALS AND METHODS

The aim of this research was to determine the effect of laser irradiation on free radical level in spring wheat grains. The material consisted of 10 Polish breeding cultivars: Alkora, Banti, Eta, Henika, Hera, Igna, Jota, Ismena, Omega and Sigma. These cultivars were irradiated using:

(I) He-Ne laser 15 mW power; the two methods - D and R were applied according to Koper and Dygdała [8], and Koper [7]. Dose D_I was $4x10^{-3}$ J/cm^2, dose D_{II} – double dose D_I, and dose D_{III} – triple dose D_I; R_I – $4x10^{-5}$ J/cm^2, dose R_{II} – double dose R_I, R_{III} – triple dose R_I.

(II) Semiconductor type laser (wavelength 670 and 810 nm) 200 mW power; doses D_I= $5x10^{-3}$ J/cm^2, D_{II}= $2.5x10^{-2}$ J/cm^2 and D_{III} =$5x10^{-1}$ J/cm^2 were applied.

EPR spectra of the control and irradiated grains were recorded at 295 K using ESP 300E Bruker and SE Radiopan spectrometers operating at X-band frequencies with 100 kHz magnetic field modulation. Li/LiF sample was used for g parameter calibration; 4-Hydroxy-TEMPO and Reckitt's ultramarine were used as standards of spin concentration; the integral intensities of the EPR signals of investigated and standard samples were compared. The quantitative EPR technique described by Jezierski *et al.* [6] was applied. After EPR measurements the samples were dried at 375 K to remove water; the spin concentrations were calculated for dry mass. In the first type of experiments 4-5 grains were inserted into the resonance cavity in the quartz tube; the second type of experiments was performed on a single wheat grain before and after irradiation.

RESULTS AND DISCUSSION

The EPR spectra observed both for control and laser irradiated wheat grains exhibit the same g parameters: strong signal at g = 2.0047 and weak one at g = 2.0057-2.0060. The first strong signal is characteristic of rich-in-oxygen (semiquinone or similar) radicals trapped in polyphenolic or melanoidin matrices according to Czechowski and Jezierski [1]; the weak signal may be attributed to a peroxy radical. Laser irradiation enhances the concentration of the radicals characterized by g = 2.0047 without changes in the g parameter.

The two experiments were carried out on the wheat grain. In the first experiment (I) the He-Ne laser was used; in the second one (II) the semiconductor laser was applied (see Experimental).

The observed concentrations of radicals are in order 10^{16} spins/gram of dry mass. Data for the control grains and after laser irradiation are given in Table 1. Application of the Student t test (Table 2) shows that the increase of the spin concentration after irradiation is statistically significant; the average increase is about 30% for the D method, and 25% for the R method. No significant dependence on the dose was observed. This phenomenon results probably from the limited amount of the „transient" radicals appearing in the sample after laser irradiation; the lowest dose is enough in this case. It is possible that during strong laser irradiation the „steady state" appears and equilibrium between formation and decay of the radicals takes place. The EPR signal of the „transient" radicals gradually decreases for several days to stabilize at the normal level of radical concentration.

The experiment (II) was proved for the single grains of the Henika cultivar.

In this case we observed the 10% increase of the radical signal independent of the dose of the laser radiation (see Experimental). The increase of free radical concentration is also statistically significant; the experimental error was less than 2% in this experiment.

Table 1. The spin concentrations for the wheat grains control and laser irradiated grains (in 10^{14} spins/gram) - Experiment I.

Cultivar	Control	D_I	D_{II}	D_{III}	R_I	R_{II}	R_{III}
Alkora	122	170	183	176	163	151	194
Banti	146	265	192	280	158	192	284
Eta	116	203	170	184	175	187	144
Henika	102	103	95	154	166	103	133
Hera	130	133	174	172	187	151	157
Igna	130	167	143	152	147	165	129
Ismena	124	144	158	137	128	151	140
Jota	132	252	155	168	132	122	153
Omega	116	155	158	199	138	210	218
Sigma	158	175	171	131	161	139	161

Table 2. The test of differences among middle values for link pairs - (Experiment I).

	D_I-control	D_{II}- control	D_{III}-control	R_I-control	R_{II}-control	R_{III}-control
N	10	10	10	10	10	10
$\sum d$	491	323	474	279	295	437
$\bar{d}$	49.10	32.30	47.70	27.90	29.50	43.70
$\sum x^2$	17914.90	4092.10	16778.10	5664.90	10988.50	18716.10
S^2	1990.54	454.68	1864.23	629.43	1220.94	2079.57
$\bar{S}_x$	14.11	6.74	13.65	7.93	11.05	14.42
t	3.48*	4.79*	3.49*	3.52*	2.67*	2.05*

* - significant at P=0.05
Experimental conditions - see text

CONCLUSIONS

Laser irradiation of wheat grains results in an increase of stable radical concentration. The signal at g=2.0046 increases 10-30%; after irradiation the signal decreases to the normal level for several days. No distinct dependence on the cultivar, dose and irradiation technique was observed. The investigations will be continued.

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