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INFLUENCE OF CADMIUM AND SELENIUM ON PHOTOSYNTHESIS ACTIVITY OF RAPE AND WHEAT PLANTS STUDIED BY EPR

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Electron paramagnetic resonance technique was used to study cadmium and selenium stress on photosystem activity of rape and wheat seedlings. It was found that leaves of healthy, not stressed plants exhibited two kinds of EPR signals. The narrow, easily saturated signal with g = 2.0026 was attributed to primary donor $P^{\bullet+}$ of photosystem PS I. The remaining lines of the spectrum, showing unusual stability, were ascribed to so-called "long lived" radicals of carbohydrate character. The supplementation of rape with cadmium led to the strong decrease of the intensity of primary donor $P^{\bullet+}$ signal, indicating the inhibition of photosynthesis processes upon cadmium stress. Simultaneously, the increase of the intensity of the lines belonging to carbohydrate radical occurred. The latter effect was connected with higher accumulation of starch in the rape leaves, confirmed by electron microscopy photographs. On the other hand, the supplementation with selenium improved the photosynthesis processes reflecting in the increase of the primary donor $P^{\bullet+}$ signal intensity in the spectrum of wheat leaves. It is suggested that starch, which is accumulated in greater amounts in plants subjected to cadmium supplementation, may act as traps for free electrons produced under stress conditions.

INTRODUCTION

It is known that during vital processes occurring in plant and animal cells the different reactive radicals are formed, which could injure the biological structures leading to serious diseases. In pathological conditions the generation of dangerous reactive species become more intense. One of such toxic agents influencing the production of free radicals in cells of living organism is cadmium, one of heavy metals being the industrial pollution. However, the mechanism of its effect on radical formation is not explained. On the other hand, it is well known that selenium plays the protective role inhibiting the generation of reactive free radicals (Filek, Kościelniak, Łabanowska, Bednarska and Bidzińska, 2010).

The aim of these studies was to investigate the influence of cadmium and selenium stress on the process of photosynthesis. Electron paramagnetic resonance (EPR) technique was used to detect the radical species, as well the metal ions appearing in raw leaves of rape and wheat before and after the supplementation of plants with cadmium and selenium salts.

MATERIALS AND METHODS

Rape (*Brassica napus* var. *oleifera*) seeds, var. Gorczanski and wheat (*Triticum aestivum* L.cv. Kamila) were germinated for 3 days at the stable temperature of 25°C. Well-germinating seedlings were transferred to dishes containing the Hoagland water nutrient (1:1, v:v) (Hoagland and Arnon 1938). Besides the control samples, Hoagland nutrient supplemented with $CdCl_2$ at a concentration of 400μM dm^{-3} (Cd media) and Na_2SeO_4 at a concentration of 2μM dm^{-3} (Se media) were used to cultivation of rape and wheat, respectively. The plants were cultivated in a growth chamber at a light intensity of 450 μmol (quanta) $m^{-2}s^{-1}$, at 20/17°C (day/night) and a 15-h photoperiod. The measurements were carried out on leaves after two weeks culture.

Electron paramagnetic resonance (EPR) spectra were redorded with an X-band Bruker Elexsys 500 spectrometer (Karlsruhe, Germany) with 100 kHz modulation at room temperature. The spectra were recorded with different microwave power: 0.03, 0.3, 3.0 and 10.0 mW. EPR parameters were found by a simulation method using the program SIM 14 (Lozos, Hoffman and Franz, 1974). The accuracy of determination of EPR parameters was: ± 0.005 and 0.0005 for g-factors of paramagnetic metal ions and radical species, respectively, and ± 0.5 and ± 0.1mT for parameters A of paramagnetic metal ions and radical species, respectively. The EPR measurements were performed for the samples of raw leaves of rape and wheat plants not stressed and stressed with cadmium and selenium, respectively.

RESULTS AND DISCUSSION

The raw leaves of rape and wheat exhibit similar EPR spectra consisting of several signals (Fig. 1). Their relative intensities depend on the kind of plant. The simulation procedure reveals that the anisotropic signal (signal A) with $g_{//}$ = 2.185 $g_{\perp}$ = 2.047 (g_{av} = 2.093) is the main component of the spectrum registered at room temperature. This signal is characteristic for square planar thiosemicarbazone complexes of Cu^{2+} (Chikate, 2005, Miller and Brudvig, 1991). The hyperfine structure arising from interaction of an unpaired electron with nuclear magnetic moment of $^{63,65}Cu$ is not visible in the spectrum, so the detailed interpretation of the signal is not possible. In spite of this, parameters of the signal can be the strong indication of the presence of thiosemicarbazone copper centres situated in plastocyanine complex.

The less intensive, well resolved signal (signal B)

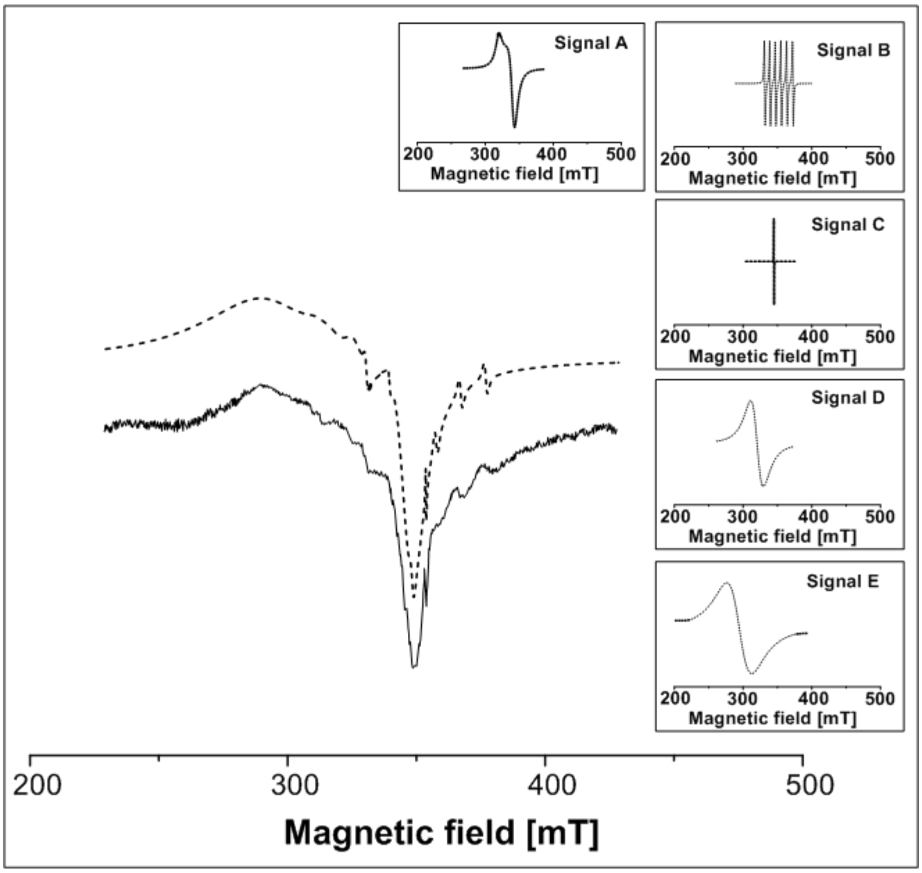

Fig. 1. The experimental (——) and simulated (------) EPR spectrum of rape leaves registered at 293 K and particular signals of simulated spectrum (inserts)

visible in the spectrum, is overlapped on the copper's one. It consists of six hyperfine lines separated by A = 9.3 mT with g = 2.003 and it is typical for manganese ions in octahedral oxide surroundings. Signals with similar parameters were observed in many biological materials and were attributed to freely rotating $[Mn(H_2O)_6]^{2+}$complex (Miller and Brudvig, 1991, Morsy and Khaled, 2002, Ogata, Murakami, Fujisawa and Kamada, 1982). Assuming that zero field splitting interactions is averaged to zero, because of rapid rotation, a superposition of fine transitions gives the single isotropic line additionally split by HFS interaction. Between the third and fourth hyperfine lines of manganese the narrow signal with g = 2.006 (signal C) is visible. The detailed analysis of the signal, performed for the spectrum registered in the range of 5 mT, reveals its more complex structure and radical character.

The good fitting of the theoretical curve to the experimental spectrum is achieved if two isotropic broad lines are added to calculated spectrum. One of them with g = 2.172 and ΔB_{pp} = 20 mT (signal D) can be attributed to freely rotating $[Cu(H_2O)_6]^{2+}$ complex. Similar signals were observed in many systems: organic (Blennow, Houborg, Andersson, Bidzińska, Dyrek and Łabanowska, 2006, Łabanowska, Bidzińska, Dyrek and Szymońska, 2006, Łabanowska, Bidzińska, Dyrek, Fortuna, Pietrzyk, Rożnowski and Socha, 2008) and inorganic (Conessa and Soria, 1979, Goslar and Więckowski, 1985). The second signal with g = 2.321 and ΔB_{pp} = 40 mT (signal E) is tentatively ascribed to Fe^{3+} ions. The lack of the line at g = 4.3 in the spectrum can be rather an indication of the presence of low spin iron species with S = ½ (Lobana and Sharma, 1989), which exhibit the g-factor values in the range of 2.0 – 3.0 (Poltoratski and Ehrenberg, 1967, Cheesman, Kadir, Al-Basseet, Al-Massad, Farrar, Greenwood, Thomson and Moore, 1992).

As it was stated earlier, the narrow signal C is present in the spectrum of raw rape and wheat leaves. The g parameter of this signal points to the radical character of the paramagnetic centre. Therefore, this signal was examined at lower microwave power (Fig. 2). The signal of rape leaf registered at 3 mW consists mainly of two doublets I and II of Gaussian character with g = 2.0062, A = 0.2 mT and g = 2.0069, A = 1.6 mT, respectively (Fig. 2a, inserts). A decrease of microwave power to 0.3 mW was accompanied by a significant decrease of the signal I and practically vanishing of the signal II (Fig 2b).

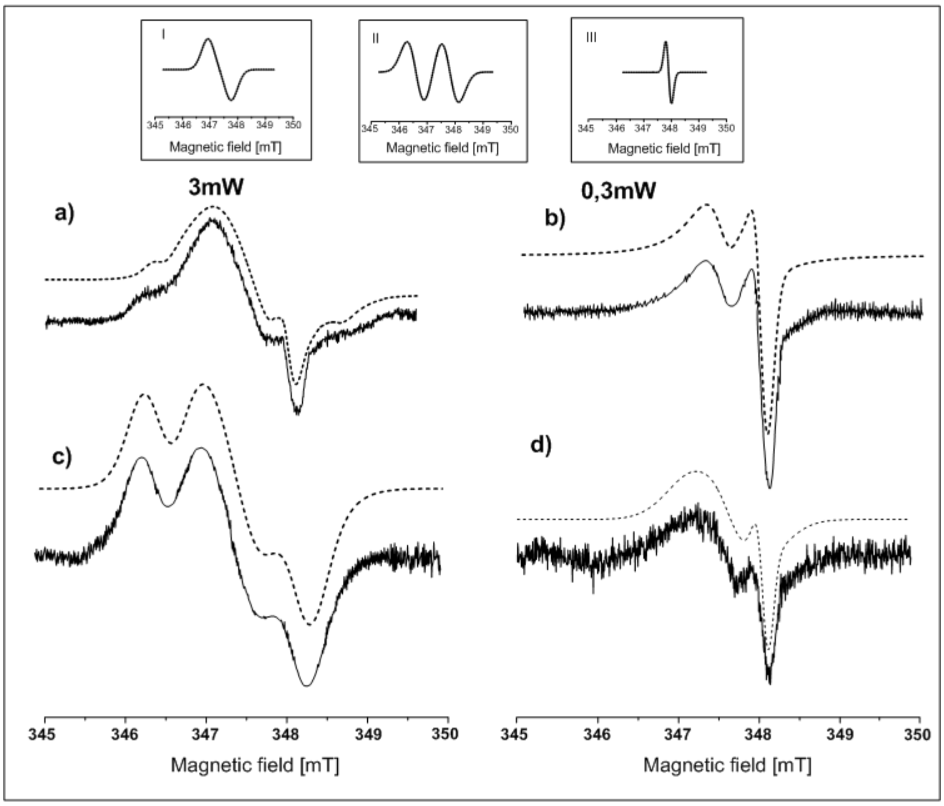

Fig. 2. The experimental (——) and simulated (------) EPR spectra of rape leaves registered at 293 K, in the range 5 mT. a, b) – the spectrum of not stressed leaves, c, d) – the spectrum of leaves stressed by cadmium inserts: particular signals of simulated spectrum

Simultaneously, with the diminishing of signals I and II, the narrow signal III also of Gaussian shape, at g = 2.0026, becomes better visible (Fig. 2b and insert). The simulation procedure reveals a small anisotropy of this signal (g_1 = 2.0033, g_2 = 2.0028, g_3 = 2.0018). Subsequent lowering of microwave power to 0.03 mW leads to the spectrum with only the signal III. The different saturation ability of these signals allows to attribute them to different radical centres. The characteristic feature of signals I and II is their high stability, whereas signal III, as it was shown in (Łabanowska, Kurdziel, Bidzińska, Filek and Kuliś, 2010) is sensitive to light. The similar spectrum is observed in the case of wheat leaves with, however, more intensive doublet I split by 0.6 mT and doublet II exhibiting also larger split equal to 1.9 mT (Fig. 3a and inserts). Additionally, the narrow signal with g = 2.0060 (signal IV) is also visible on hyperfine structure background, between the lines of signal I (Fig. 3a and insert). The diminishing of microwave power reveals the signal III, at g = 2.0026, however, with lower intensity than that observed in the spectrum of rape leaves (Fig. 3b).

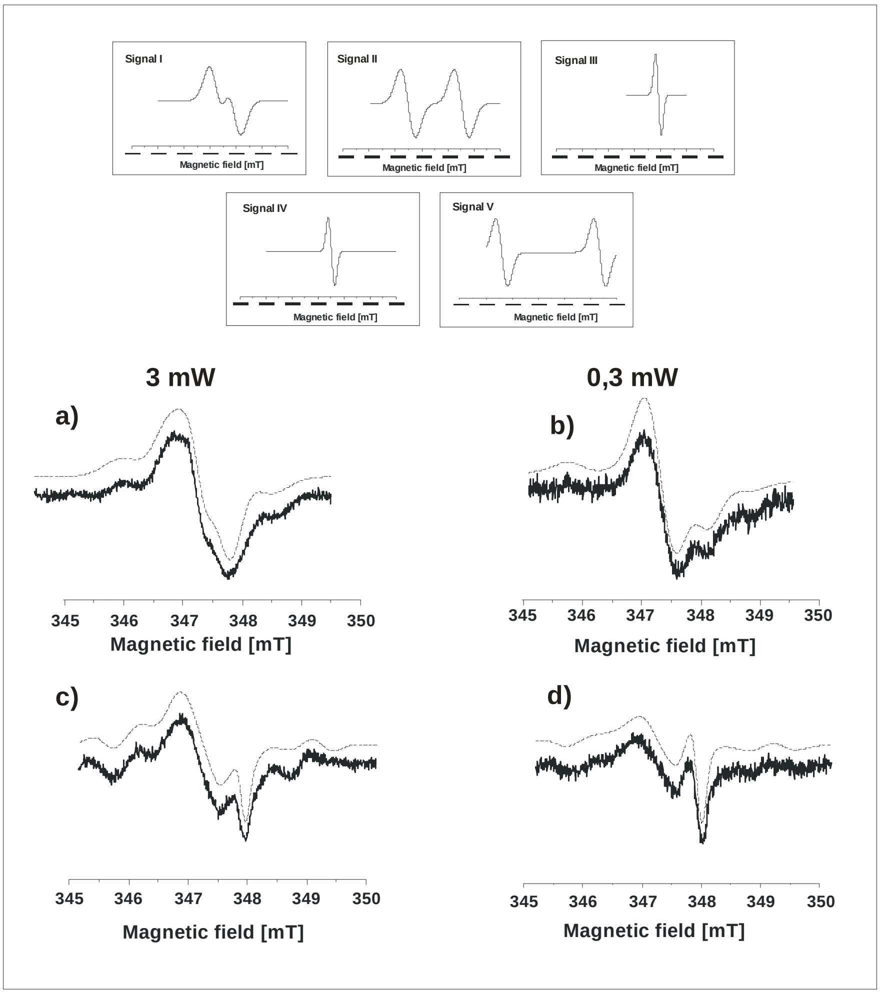

Fig. 3 The experimental (——) and simulated (------) EPR spectra of wheat leaves registered at 293 K, in the range 5 mT. a, b) – the spectrum of not stressed leaves, c, d) – the spectrum of leaves stressed by selenium, inserts: particular signals of simulated spectrum

The supplementation of the rape with cadmium salt leads to significant changes in the EPR spectrum. Doublets I and II become better separated and their intensities increase (Fig. 2c). These changes are accompanied by the decrease of the intensity of the signal III (Fig. 2d). The supplementation of the wheat with selenium also causes the changes in EPR spectrum (Fig. 3c). The integral intensity of the spectrum slightly increases, the narrow lines at g = 2.0060 (signal IV) disappears from the spectrum, whereas the split of the doublet I decreases to 0.3 mT. Additional lines separated by 3.6 mT (signal V) become visible and the signal III, more distinctly isolated in the spectrum registered at lower (0.3 mW) microwave power, strongly increases its intensity (Fig. 3d). Its EPR parameters (g_1 = 2.0033, g_2 = 2.0026, g_3 = 2.0019) and the facility to undergo saturation are similar to those of the signal III in the spectrum of not stressed rape.

The EPR characteristics of particular signals observed in the spectra of stressed and not stressed leaves allows to ascribe them to definite species. The main feature, which differentiates the radical signals is their ability to undergo the saturation. The signal with g_{av} = 2.0026 (signal III), easily saturating, not exhibiting the HFS structure in X-band, was described in literature as originating from a primary donor $P^{\bullet+}$ of photosystem PS I (Käss, Fromme, Witt and Lubitz, 2001, Miller and Brudvig, 1991, Norris, Uphaus, Crespi and Katz, 1971). This signal is present in raw green leaves and its intensity strongly depends on the degree of lighting and decreases upon darkness (Łabanowska et al., 2010). The remaining signals in the spectra (signals I, II and V) are doublets with characteristic splits 0.2-0.6 mT, 1.4 -1.9 mT and 3.6 mT. The g factor values of the signals, exclude ascribing them to inorganic oxygen based radicals. On the other hand, the high stability of the species indicates their bonding to the compounds with high molecular weight, or their immobilization in specific cell structures. It is known that many carbohydrate radicals created in mono or polysaccharides exhibit the high stability, therefore, such molecules, being the products of photosynthesis, are taken into account as the place of the creation and stabilization of long lived radical species. These species are probably created by the abstraction of hydrogen or OH^- group from carbon atom of the carbohydrate molecule. A magnetic moment of an unpaired electron situated at carbon atom of carbohydrate can interact with nuclear spin of adjacent hydrogen atoms leading to the appearing of the hyperfine structure. The values of splits observed in the spectra of the rape and wheat leaves are lying in the range of those found for different carbohydrates (Abagyan and Apresyan, 2002, Kuzuya, Yamauchi and Kondo, 1999, Madden and Bernhard, 1982, Vanhaelewyn, Sadło, Callens, Mondelaers, De Frenne and Matthys, 2000, Vanhaelewyn, Lahorte, De Proft, Mondelaers, Geerlings and Callens, 2001). However, it is worthwhile to notice that X-band is not sufficient to observe all, well resolved hyperfine lines. Therefore, it is difficult, for complex biological system, to indicate exactly the carbon atom at which an unpaired electron is localized. For simpler, better defined structure of carbohydrates the localization of radicals is found at various carbon atoms (Vanhaelewyn et al., 2001, Madden and Bernhard, 1982, Kuzuya et al., 1999, Dyrek, Bidzińska, Łabanowska, Fortuna, Przetaczek and Pietrzyk, 2007, Łabanowska et al., 2008). In spite of these differences, the EPR characteristics of reported signals is similar and close to that found for species observed in raw leaves. On this basis, the signals I, II and V, exhibiting HFS can be treated as originating from carbohydrate radicals.

The main difference between the spectra of both, not stressed rape and wheat leaves, is the presence of the single line – signal IV in the spectrum of wheat leaves. The earlier investigation of wheat leaves did not manifest such signal (Łabanowska et al., 2010).

During supplementation of rape with cadmium the concentration of carbohydrate radicals increases. As it was stated in (Filek et al., 2010), this increase was accompanied with starch accumulation in rape leaves, found by microscopic observation. Simultaneously, the decrease of the intensity of the cation radical $P^{\bullet+}$ signal is observed, confirming the inhibition of photosynthesis by cadmium addition. The increase of the content of stable carbohydrate radicals, probably localized in starch matrix, strongly suggests the generation of free electrons in redox processes, influenced by cadmium. In view of this, the starch matrix can be considered as a trap of electrons induced during cadmium stress.

Contrary to the cadmium influence, the selenium addition leads to the increase of the cation radical $P^{\bullet+}$ signal intensity in the spectrum of wheat leaves indicating improvement of photosynthesis efficiency. Simultaneously, the single signal IV vanishes. Taking into account, that this signal is not present in the spectrum of not stressed rape leaves in which $P^{\bullet+}$ signal is more intensive, as well in other samples of wheat leaves (Łabanowska et al., 2010) the appearing of the signal IV can be caused by some disturbances of photosynthesis process in not stressed wheat leaves. Supplementation with selenium leading to amelioration of photosynthesis eliminates the signal IV.

CONCLUSIONS

The EPR method is very useful to follow the photosynthesis processes occurring in raw plants, as well to estimate the plant condition.

The presence of the intensive signal of primary donor $P^{\bullet+}$ of photosystem PS I is the indication of the effective photosynthesis process. The decrease of the intensity of this signal observed under the influence of cadmium is a proof of the inhibition of photosynthesis. Free electrons which are produced during cadmium stress are trapped by carbohydrate molecules, probably localized in polymeric starch matrix, leading to the formation of stable, carbohydrate radicals. Selenium effect is connected with the activation of photosynthesis which is reflected in EPR spectrum by the increase of the signal intensity of cation radical $P^{\bullet+}$.

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