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Concentration of free radicals in pea seeds after pre-sowing treatment with magnetic field**

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A b s t r a c t. The aim of the research was the estimation of seed lot magnetic stimulation effect on free radicals concentration and dynamics of pea seeds germination. The first factor were two cultivars of pea: Rola (traditional form) and Piast (afila type), the second factor were the 3 exposure doses of magnetic field intensity: D0 – no stimulation (control), D1 – 10 750 Jm^3s and D2 – 85 987 Jm^3s. The stimulated seeds of both pea forms showed faster uptake of water and achieved larger mass during the seed imbibition in comparison to the seeds without stimulation. It was found that earlier and more steady emergence of these plants was a consequence of this process. Pea seedlings which were grown from stimulated seeds achieved in the following successive dates of measurement significantly larger hypocotyl and roots length in comparison to the seedlings without stimulation with magnetic field. A significant increase of free radicals concentration was observed in the seeds which were pre-sowing treated with magnetic field. There was no significant effect of the stimulation on the free radicals concentration in the organs of young pea plants grown from seeds of the varieties under examination. Free radicals numbers in the leaves, stems and roots did not differ significantly from those which were found in the particular organs of plants which were grown from non-stimulated seeds. The laboratory tests did not show any larger number of free radicals in the seeds harvested from the plants which were grown from the stimulated seeds.

K e y w o r d s: Electron Paramagnetic Resonance, free radicals, pea, stimulation of seeds

INTRODUCTION

Results of studies on magnetic field found in literature concern most often of effects measured as the quality of germination (Bovelli and Bennici, 2000; Chao and Walker, 1967; Hirota *et al*., 1999; Pietruszewski, 1999b) or yield increase (Phirke *et al*., 1996; Pietruszewski, 1999a; Podleśny *et al*., 2004; Vakharia *et al*., 1991). There is a lack of research which are going to explain of seeds magnetic stimulation mechanism. As is well known, the way of magnetic field action on crops seed lot have not been exactly known and explained, so far. The hypotheses which explain this phenomenon are based on fragmentary studies (Liboff, 1969; Tugulea, 2000). It is supposed that magnetic field influences the structures of cell membranes and in this way increases their permeability and ion transport in the ion channels, which then affects some metabolic pathways activity (Labels, 1993). At present it is assumed that seeds treated by magnetic stimulation show higher activity of enzymes which control the particular stages of seed germination (Aksyonov *et al.,* 2000; Lebiediev, 1975; Maling and Weissbluth-Jacobs, 1965). An important factor in the magnetic field effect on live organisms is water which is necessary for all vital processes and is very sensitive to magnetic field influence, even if it is a field with low energy (Wojtusiak and Majlert, 1992). Also the magnetic fields which are present in every living organism can play a considerable role. The occurrence of this kind of fields with low intensity was found in plant seeds, too. It is supposed that magnetic field, like some different physical factors, can effect biological objects by non-compensated electron spins – free radicals, liquid crystals or mobile electrical charges (Wardas, 1978). Free radicals, as chemically very active

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particles, enter into fast reactions with oxygen and this way they can cause changes in the course of some biochemical and physiological processes in seeds.

The aim of the research was the estimation of seed lot magnetic stimulation effect on free radicals concentration and on the dynamics of pea seeds germination.

METHODS

The experiment was conducted at the Department of Fodder Plant Cultivation, Institute of Soil Science and Plant Cultivation in Puławy. The analyses concerning free radicals estimation in seeds and plants were done by using the Electron Resonance Paramagnetic method (Schwartz, 1972; Symons, 1978) at the Experimental Physics Department, UMCS in Lublin. Seeds stimulation was done at the Physics Department, University of Agriculture in Lublin, using of a device for pre-sowing treatment of seeds with magnetic field. The first factor were two varieties of pea: Rola – the traditional type, and Piast – the afila type; the second factor were the 3 exposure doses of magnetic field intensity: D0 – without stimulation (control), D1 – 10 750 and D2 – 85 987 J m^3 s.

The number of free radicals was estimated three times: 1) in the seeds – directly after stimulation and before sowing, 2) in the plants which were grown from the stimulated seed lot in the first period of their development and 3) in the seeds from these plants after harvest. Therefore, some of the seeds after stimulation was analyzed by the EPR method (Electron Paramagnetic Resonance) and the next part of these seeds portion were sown in Mitscherlich pots with the aim of getting material to analyze over the remaining harvest period. Measurements of EPR spectra were made with EPR spectrometer type SE/X–2547 resonance bay type CX-101TE_{102}. Analyzed seed lot was placed in a test-tube with thin wall, precise made from synthetic quartz (733-5PQ –7 WILMAD). Synthetic quartz allows to pass the ultraviolet radiation to 200 nm and facilitates measurement of weak EPR signals. The EPR spectrums were recorded at the following settings: microwave frequency 9.4-9.5 GHz, microwave power about 15 mW, modulation frequency 100 kHz with amplitude 0.5 mT, times constant 1s and brooming 20 mT 4 min^{-1}. At EPR measurements microwaves were used with wavelength of $\lambda = 3\ 10^{-2}$ m in magnetic field of about 340 mT. Weak pitch EPR sample (9004450-02, 3.3 10^{-4}% pitch in KCl) was used as a standard for the delimitation of free radicals concentration. The free radicals concentration was delimitated by comparison of standard spectrum to seeds spectrums after stimulation by a dose of magnetic field. In the end, the concentration of free radicals was calculated on 1 g mass of seeds. The experiments were conducted at room temperature.

RESULTS

During the tests, differentiated concentration of free radicals in the seeds of both pea varieties was observed. In the seeds of the Piast variety, a considerably greater number of free radicals was found than in the seeds of the Rola variety (Fig. 1). In 1 g of Piast pea seeds there was an average of 2.62 10^{14} and of the Rola variety 1.77 10^{14} of free radicals. Also, distinct differences were found in free radicals concentration in samples treated and non-treated with magnetic field. The concentration of free radicals was increasing together with increase of magnetic field exposure dose. After the Piast variety seeds stimulation with doses D1 and D2, the number of free radicals increased, in comparison to seeds without stimulation, by 36 and 84.2%, respectively, and in the case of the Rola variety – by 66 and 75.1%, respectively.

The measurements taken at a later date, when the plants were in the beginning stages of development (the phase of 3-4 leaves), showed that free radicals concentration in the studied pea organs was differentiated (Table 1). The highest

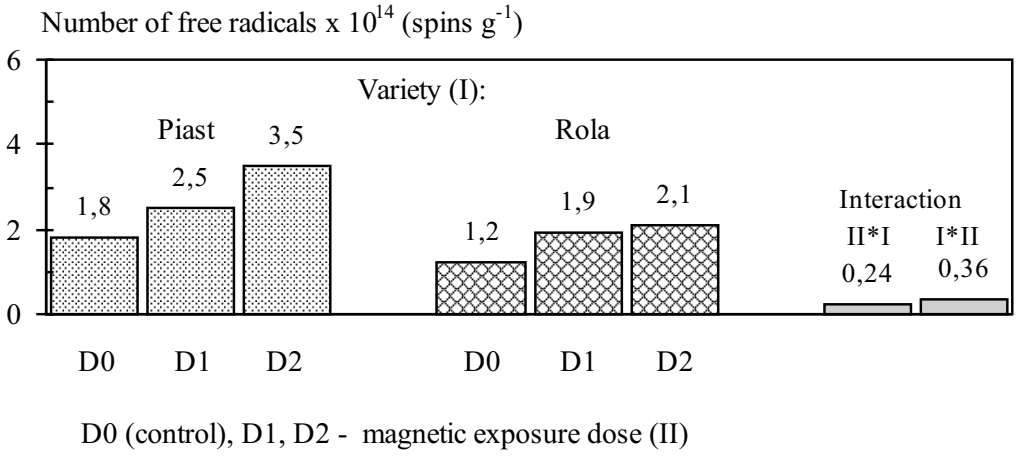

Fig.1. Number of free radicals in pea seeds directly after treatment with magnetic field.

number of free radicals was found in leaves, and the lowest in seeds. Besides, a greater number of free radicals was found in the seeds and at roots of the Piast variety in comparison with the same organs of Rola plants variety. However, no significant effect of pre-sowing treatment of seeds with magnetic field was observed on free radicals concentration in the aboveground parts/organs of plants grown from stimulated seeds. The same situation was observed in the case of roots. Therefore, the measurements of free radicals concentration in seeds collected from plants grown from stimulated seed lot showed that seeds of pea var. Piast are characterized by their greater number than seeds of Rola variety. No significant effect of magnetic field on free radicals concentration was found in seeds collected from plants grown from stimulated seed lot. The number of free radicals in 1g of Piast variety seeds was 1.74 10^{14} and in those of the Rola variety 1.04 10^{14}, and it was similar to the concentration in seeds before their sowing.

The dynamics of seed mass increase during the germination period was similar for both studied pea varieties and the course of its changes as a mean for the two pea varieties is shown in Fig. 2. Pre-sowing treating of seed lot with magnetic field positively affected the mass of both pea varieties seeds during their imbibition. The greatest mass of seeds was achieved after 24 to 48 h after sowing. Pea seeds treated with dose D1 of magnetic field increased their mass, in comparison with control seeds after 12, 24 and 48 h from sowing, by 8.8, 13.5 and 15.2%, and at dose D2 by 11.5, 18.9 and 21.7%, respectively. The mean mass of one pea seed in the period from sowing to germination was 37g and 34 g for the Piast and Rola varieties, respectively.

Faster and more uniform germination of seeds treated with magnetic field in comparison to control seeds was observed after sowing. The differences in the course of germination were visible after 24 h from sowing, but the greatest differentiation of seed germination dynamic of both pea varieties was shown during 24 to 96 h after sowing. Control seeds and seeds treated with magnetic field completed the germination process after 170 and 144 h from sowing, respectively. No distinct differences were observed in the germination dynamics of the studied pea varieties. The course of germination dynamics for D1 and D2 doses was

T a b l e 1. Number of free radicals in plant organs grown from seeds treated and non-treated by magnetic field x 10^{14} (spins g^{-1})

Description	Stems	Leaves	Roots	Seeds
Pea variety:	2.28a*	3.52a	1.71a	1.74a
Piast	2.34a	3.41a	1.48b	1.04b
Rola				
Magnetic exposure dose:	2.39a	3.48a	1.51a	1.44a
D0	2.24a	3.42a	1.72a	1.38a
D1	2.30a	3.51a	1.53a	1.35a
D2				

* Numbers in columns denoted with the same letters do not differ significantly.

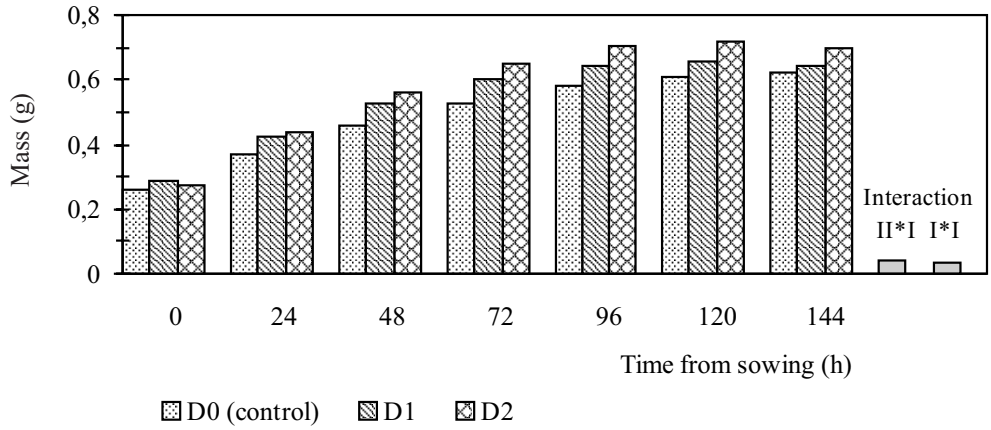

Fig. 2. Dynamics of seeds imbibed mass and pea seedlings mass changes in dependence on magnetic field dose.

very similar, so it is shown as common regression curves for both doses (Fig. 3). After 144 h from sowing all seeds reached 100% of germination capacity.

Seed stimulation with magnetic field had a modifying effect on initial growth and development of pea seedlings, mostly on the length of roots and hypocotyls (Figs 4 and 5). The greater exposure dose had a more pronounced effect on the length increase of the studied plant organs than the lower one. Both magnetic field doses significantly affected the length increase of stems and roots of the pea varieties, but better effects were achieved after seed treatment with the higher dose. The increase of the Piast variety root and stem length after 6 days from sowing was 13.4 and 14.5% for objects treated with both doses of magnetic field and for control, respectively, while for the Rola variety these values were 11.2 and 12.2%, respectively. Roots and stems of the Piast variety seedlings had the same length as seedlings of the Rola variety.

As well as the length of roots and stems of the seedlings, their mass was changing in dependence on the dose of magnetic field used (Figs 6 and 7). After 144 h from sowing, both doses of magnetic field caused an increase of Piast pea roots and stems mass in comparison to the control object – by 15.2 and 15.6%, respectively. For the Rola variety, these parameters had the following values: 12.9 and 15.3%, respectively. Roots and stems of the Piast pea seedlings gave greater mass than those of the Rola variety.

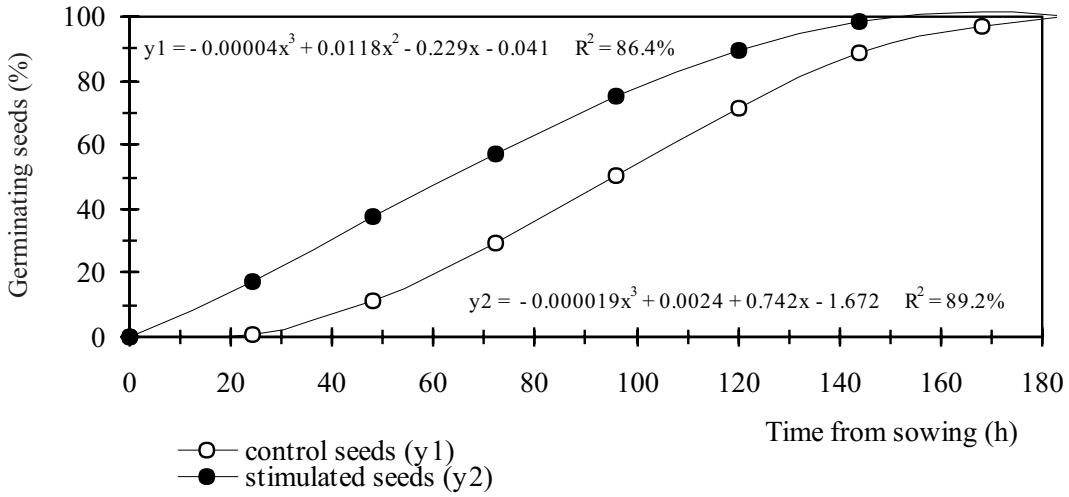

Fig. 3. Dynamics of pea seed germination in dependence on magnetic field action expressed in percentage of germinating seeds.

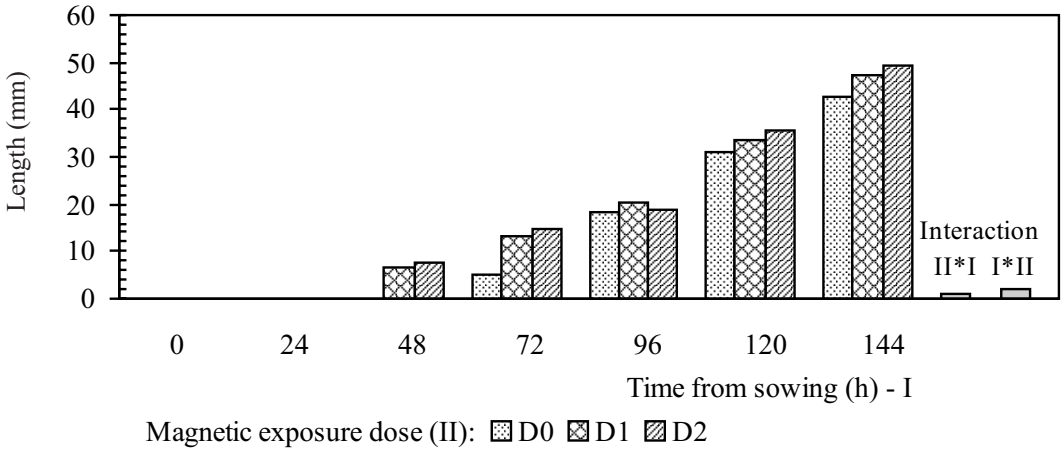

Fig. 4. Length of pea seedling roots grown from seeds treated and non-treated with magnetic field.

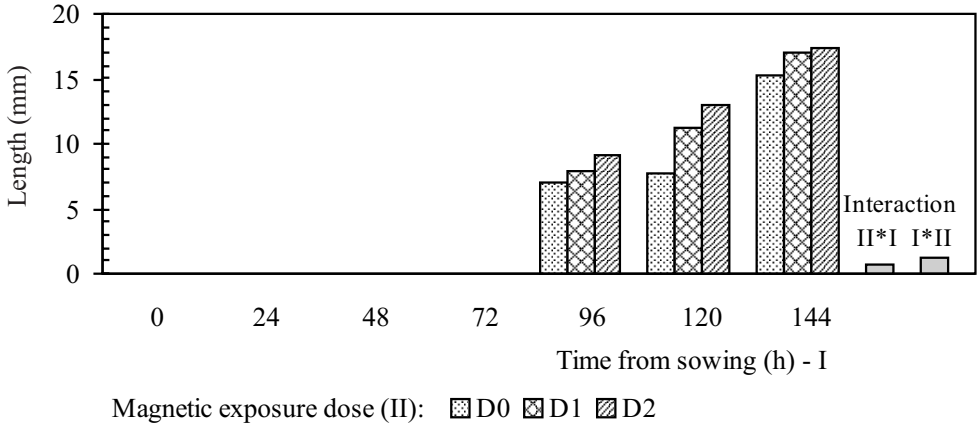

Fig. 5. Length of pea seedling stems grown from seeds treated and non-treated with magnetic field.

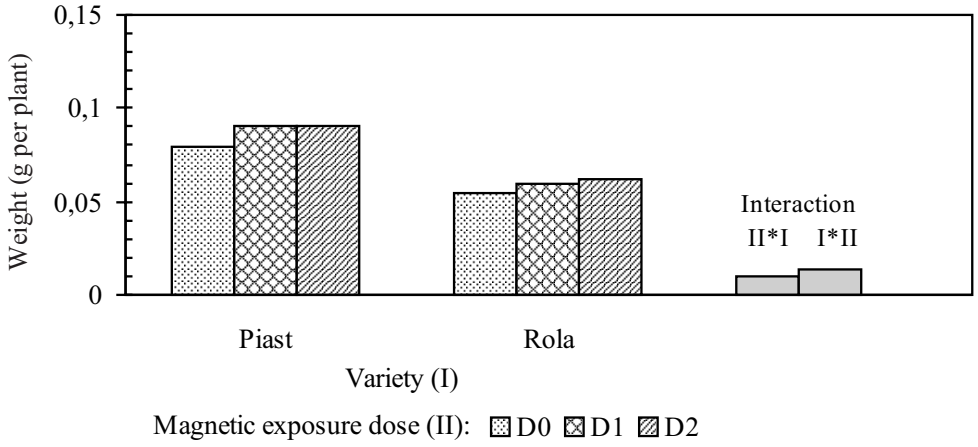

Fig. 6. Weight of pea seedling roots after 144 h from sowing.

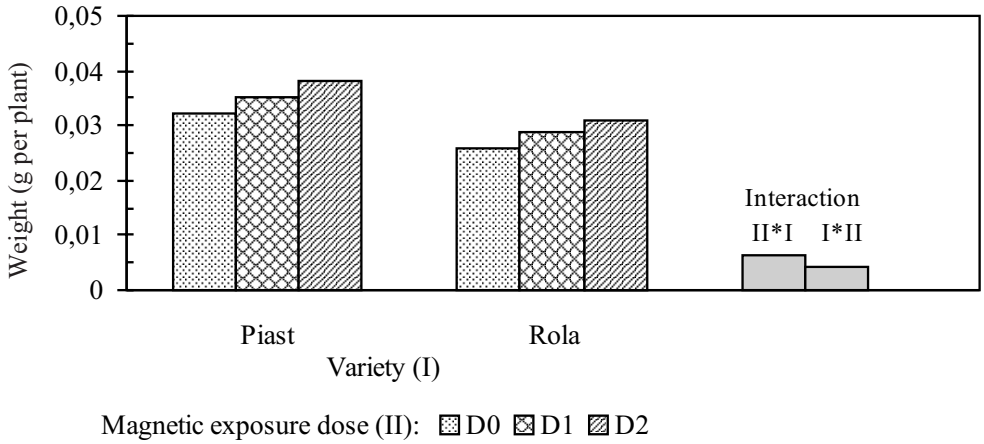

Fig. 7. Weight of pea seedling stems after 144 h from sowing.

DISCUSSION

The study showed a significant increase of free radicals concentration in seeds which were treated with magnetic field prior to sowing. The concentration of free radicals in seeds depended on magnetic field exposure dose and on pea variety. A greater number of free radicals was found in seeds treated with the higher dose of magnetic field in comparison to the lower one. Seeds of the Piast variety had a greater concentration of free radicals than seeds of the Rola pea variety. The calculated values of g which means the spectroscopy diffusion factor were for – both studied pea varieties – in the range from 2.0044 to 2.0049. However, factor g for free electrons is 2.0023. Comparing the values of both factors we can conclude that stable free radicals in the seeds are more massive and probably have more limited freedom of movement in comparison to free electrons. Because the value of factor g in pea seeds showed no changes before and after the treatment, it should be supposed that free radicals contained in the seeds have similar structure in spite of magnetic field treatment.

Pea seeds are valuable fodder for farm animals (Pastuszewska, 1997) and a valuable element of human diet (Lampart-Szczapa, 1997). The increase of free radicals concentration in plant material and in the seeds of plants grown from seed lot treated with magnetic field could considerably reduce the possibility of use of the material for nutritional purposes (Slater, 1989). However, as a result of detailed laboratory studies of plant material, no significant increase of free radicals number was found in the particular organs of aboveground part of plants, nor in the roots. It should be added that this material was collected in the early period of plant development. It is supposed that young plants have the greatest concentration of free radicals. Spectroscopy tests showed that concentration of free radicals in the seeds collected from plants grown from stimulated seed lot is almost the same as in the seeds originating from the control, and similar to free radicals concentration in the control seeds before their sowing.

As a result of magnetic field effect on seeds, some changes in the initial period of plant development were observed. Young seedlings grown from seeds stimulated with magnetic field developed longer hypocotyl and roots. They were characterized also by greater vigour which is sometimes called the 'growth power of plants' and which decides about the dynamics of growth and development of seedlings and, ultimately, determines the plant yielding. The statements of the authors concerning this problem have found the confirmation in an earlier study of Pittman *et al.* (1977) and Podleśny *et al*. (2003) in reference to the growth and development of some cereals and legumes species. A consequence of the accelerated seed metabolism and changes in the course of biochemical and physiological processes in seeds resulting from their treatment with magnetic field was faster germination of pea seeds. Most papers dedicated to these problems show a favourable effect of magnetic field on the germination and emergence of cereals (Pietruszewski, 1999b) and legumes (Podleśny, 2003). The studies by Prokop e*t al*. (2002) prove also a positive effect of magnetic field on the germination of some vegetables. At present, the predominant opinion is such that physical factors affect in the greatest degree the initial growth and development of plants, and particularly their germination and emergence. An example can be the paper of Podleśny *et al.* (2004), concerning magnetic stimulation of white lupine seeds. Studies conducted by Rochalska (2002) on the germination and growth of wheat, triticale, maize and soybean, also indicate that magnetic field can be used as a method of seed vigour improvement. It is particularly important when the weather conditions are unfavourable for germination, or in the case of seeds with inferior quality parameters. The value of obtained effects was dependent on the dose of magnetic field and on the time of exposure used. It is known from literature that the best effects of seeds stimulation with physical factors are possible to obtain when optimal exposure doses are applied. Lower doses of a factor usually stimulate seed germination and later development of plants grown from them, while higher doses can have disadvantageous effects and sometimes can even cause mutations. The experiment with pea varieties indicated that both doses of magnetic field used in the experiment had a favourable effect on the studied features.

The conducted research showed a positive effect of magnetic stimulation of seeds on the increase of pea hypocotyl and root length. Therefore, we can testify that the changes under discussion which occur in irradiated seeds cause then some changes in the development dynamics of plants grown from them. It confirms also the opinion that the greatest changes occur in the stimulated seeds and in the initial period of plant development; these changes lead to faster growth of plants in the later period of their development. Pea seeds treated with magnetic field faster increased their mass in the imbibing period in comparison to control seeds, probably in consequence of faster water uptake. The research conducted by Grzesiuk and Rejowski (1957) showed that maize seeds which were treated, before sowing, with ultrasound waves took up decidedly more water than control ones. The acceleration of seed respiration and increase of water uptake was observed also after the use of ionizing radiation. Although ultrasounds as well as ionizing radiation have a different nature than magnetic field, their influence on seed lot of crops can be similar.

CONCLUSIONS

1. A significant increase of free radicals concentration was found in pea seeds treated with magnetic field prior to sowing. The greatest increase of their number was found after treating the seeds with magnetic field of 85 mT. The seeds of the Piast pea variety contained more free radicals than the seeds of the Rola variety.

2. Pea seeds subjected to magnetic field showed greater dynamics of mass increase during imbibition than control seeds as a result of faster water uptake.

3. Treating seeds with magnetic field had a positive effect on growth and development of seedlings. The young faba bean plants grown from stimulated seed lot were characterized by longer hypocotyl and primary roots in comparison to seedlings grown from non-stimulated seeds.

4. No significant effect of seed stimulation on free radicals concentration in the roots and aboveground parts of pea plants was found. The number of free radicals did not differ significantly from that which was found at the same organs of plants grown from non-stimulated seeds. Spectroscopic tests did not show any increase in the number of free radicals in seeds collected from plants grown from stimulated seed lot.

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