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Concentration of free radicals in faba bean seeds after the pre-sowing treatment of the seeds with laser light**

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A b s t r a c t. Experiments were conducted at the Institute of Soil Science and Plant Cultivation in Puławy, the Agricultural University and University of Maria Curie-Skłodowska in Lublin. The first row factor were three morphologically differentiated white lupine varieties: Nadwiślański (traditional form) and Tim (determinated form), and the second one - doses of laser irradiation: *D*0 - without irradiation, *D*1 - single fold irradiation, *D*2 - double irradiation, *D*3 - three fold irradiation, *D*4 - four fold irradiation, *D*5 - five fold irradiation. Single exposition was equal to $4 \cdot 10^{-3}$ J $cm^{-2}s^{-1}$. Irradiation of double have been carried out at the Physics Department of the Agricultural University in Lublin, using a device for the laser irradiation of seed prior to sowing. The irradiated seeds of both faba bean forms gave a faster uptake of water and achieved a larger mass during seed imbibing in comparison to seeds without irradiation. It was found that the earlier and steadier emergence of these plants was a consequence of this process. Faba bean seedlings which were grown from irradiated seeds achieved, in the succeeding measurement dates, significantly larger coleoptile and root length in comparison to those seedlings which had not been so irradiated by laser light. A significant increase was observed in the concentration of free radicals in those seeds which were treated, before sowing, with laser light. The largest increase in the number of free radicals in the seed of both faba bean varieties was found after three and four fold seed irradiation. There was no significant effect of examined varieties in seed irradiation on free radical concentration in the young faba bean plant organs. The number of free radicals in the leaves, stems and roots did not differ significantly from that found in the particular organs of plant grown from non-irradiated seeds. Laboratory research did not show any increase in the number of free radicals in seeds harvested from plants grown from irradiated seeds.

K e y w o r d s: laser bio-stimulation of seeds, faba bean, free radicals, Electron Paramagnetic Resonance

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INTRODUCTION

Irradiation of seeds by laser influences modifies the development and yield of cultivated plants [8,12,18]. In recent years, increases in the importance of the physical factors of the treatment of pre-sowing seeds in connection with tendencies in cultivation technology, tend towards the propagation of plant production methods which are friendly to the environment. In the national and foreign bibliography there are many papers proving the favourable effect of laser light on the size of yield [2,9,13,14], and sometimes also, on quality [1,5,10].

Among them is a lack of any detailed particular research results showing changes in the irradiated seeds and plants grown from these seeds. Among other things are hypothesis that laser light can increase the number of free radicals in the seeds, which can react with oxygen and this process leads to the formation of peroxides. The activity of hydrolitic enzymes increases under the influence of these changes and fast mobilisation of reserve substances increases too, which accelerates plant emergence and development.

The aim of the research was to determine the influence of the treatment with laser light of seeds at the pre-sowing stage and the changes in the seeds, especially on the concentration of free radicals in the seeds and the faba bean plants.

METHODS

An experiment was carried out in the Forage Plant Cultivation Department, Institute of Soil Science and Plant Cultivation in Pulawy. Analyses concerning the estimation of the number of free radical in the seeds and plants were done by the Electron Paramagnetic Resonance method [16,17] at

the Experimental Physics Department, Marie Curie-Skłodowska University in Lublin. Seed irradiation was done at the Physics Department of the Agricultural University in Lublin, by using a special device for the pre-sowing treatment of seeds by laser light. The first row factor were two faba bean genotypes: Nadwiślański - a traditional variety and Tim - determinate form, and the second row factor were 5 doses of laser irradiation: *D*0 - without irradiation, *D*1 - single irradiation, *D*2 - double irradiation, *D*3 - three fold irradiation, *D*4 - four fold irradiation, *D*5 - five fold irradiation of seeds. Single exposition was equal to $4 \cdot 10^{-3}$ J $cm^{-2}s^{-1}$.

The number of free radicals was estimated: in the seeds directly after irradiation and before sowing, in the plants which were grown from the irradiated seed lot in the first period of their development and in the seeds from these plants after harvest. Therefore some of the seeds after irradiation was analysed by the EPR method (Electron Paramagnetic Resonance) and the next part of this portion of seeds were sown in Mitscherlich pots with the aim of getting material to analyse over the remaining harvest period. The measurement of the EPR spectrum was realised using a spectrometer of the EPR SE/X-2547 type with resonance bay, $CX\text{-}101TE_{102}$ type. The seed lot studied was placed in a thin-walled test tube made of synthetic quartz (733-5PQ-7 WILMAD firm). The suprasil permitted the passage of ultraviolet radiation to 200 nm and facilitated the measurement of the weak EPR signals. The registration of the EPR spectrums was conducted by: microwaves frequency 9.4 - 9.5 GHz, microwaves power about 15 mW, modulation frequency 100 kHz about amplitude 0.5 mT, time constant 1 s and brooming 20 mT $4\ min^{-1}$. In the EPR measurements, microwaves on a wavelength of $\lambda = 3 \cdot 10^{-2}$ m were used in the magnetic field more or less 340 mT. For the calculation of the concentration of free radicals, the standard used was Weak pitch EPR sample (904450-02, $3.3 \cdot 10^{-4}$% pitch in KCl). The concentration of free radicals was calculated using the comparison of a standard sample spectrum with seed spectrums after irradiation with an adequate dose of laser radiation and then the concentration was re-counted with 1 g of seeds mass. The experiments were conducted at room temperature, at 4 replications. In the statistical analysis Tukey was used half-interval of confidence, at significance level $\alpha = 0.05$.

RESULTS

In the seeds of both faba bean varieties a differentiated concentration of free radicals was observed in the experiments carried out. Almost twice the number of free radicals were found in the seeds of the Tim variety than were found in the seeds of the Nadwiślański variety.

In the 1g of the Tim variety, the seeds had an average $7.0 \cdot 10^{14}$ spins and in the Nadwiślański variety an average $4.5 \cdot 10^{14}$ spins of free radicals. Distinct differences were also found in the concentration of free radicals in those samples irradiated by laser light as opposed to those not so irradiated. This concentration increased up to the maximum value at differ laser irradiation doses for both varieties and subsequently decreased (Fig. 1). The number of free radicals of the Tim variety of seeds treated by doses of one, two, three, four and five fold irradiation increased in comparison to seeds without irradiation respectively by: 22.9, 44.1, 48.6, 36.8, 24.4% and with regard to the Nadwiślański variety of seeds respectively by: 31.5, 77.2, 68.3, 45.1 and 49.8%. The measurements carried out at the later stage, when the plants were at the first phase of development - the 2-3 leaf phase showed that the concentration of free radicals in the organs under examination was differentiated (Figs 2 and 3). Besides, at above-ground level and in the roots of the Tim variety, a larger number of free radicals was found than was found in the Nadwiślański variety. The average number of free radicals for irradiated and not irradiated seeds of the Nadwiślański variety at above-ground level and in the roots was respectively: $1.7 \cdot 10^{14}$ and $3.1 \cdot 10^{14}$ spins $\cdot g^{-1}$, and Tim variety respectively: $3.2 \cdot 10^{14}$ and $4.1 \cdot 10^{14}$ spins$\cdot g^{-1}$. No significant effect was detected of the irradiation of pre-sowing seeds on the concentration of free radicals in the above-ground part or the roots of the faba bean seedlings which were grown from

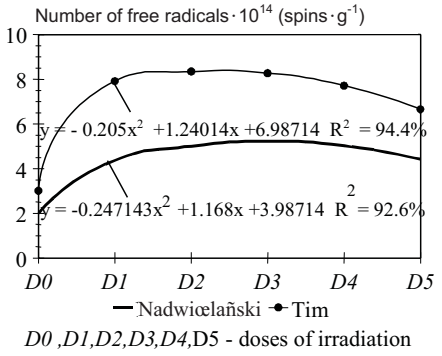

Fig. 1. Number of free radicals in faba bean seeds treated and not-treated by laser light.

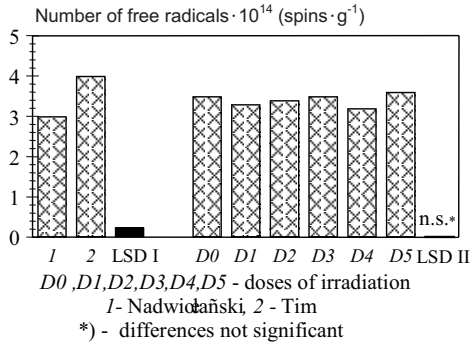

Fig. 2. Number of free radicals in faba bean roots harvested from plant grown from seeds treated and not-treated by laser light.

irradiated seeds. Measurements of the concentration of free radicals in the seeds which originated from plants grown from the irradiated seed lot show that the seeds of the Tim variety have a larger number of free radicals than those of the Nadwiślański variety (Fig. 4).

No significant irradiation effect on the concentration of free radicals in seeds collected from plants originating from irradiated and not irradiated seeds was found. The number of free radicals in 1g of the Nadwiślański variety of seed was $3.4 \cdot 10^{14}$ and in the Tim variety $6.1 \cdot 10^{14}$ spins and was similar to the seeds before sowing without irradiation.

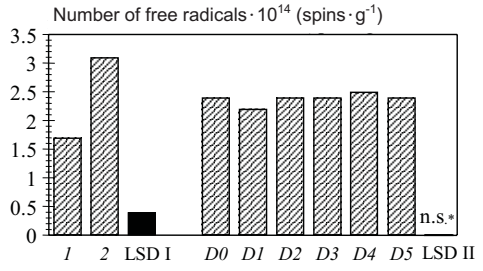

Fig. 3. Number of free radicals in the faba bean above-ground part harvested from plants grown from seeds treated and not-treated by laser light. Explanations as in Fig. 2.

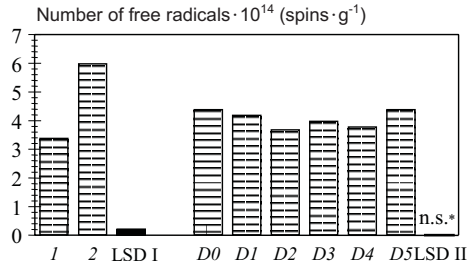

Fig. 4. Number of free radicals in the faba bean seeds harvested from plant grown from seeds treated and not-treated by laser light. Explanations as in Fig. 2.

The increase of the imbibing seed mass and the seedlings was similar in both the faba bean varieties studied, and the course of these changes is shown on the Fig. 5 as an average for two varieties. Irradiation on the pre-sowing seed lot which has a favourable influence on the mass of imbibing seeds was found from 24 to 48 h after sowing. The three fold irradiated faba bean seeds had, after 12, 24 and 48 h of being sown, increased their mass in comparison to seeds which had not been irradiated, respectively by: 48.2, 28.4, 21.9% and five fold dose respectively by: 52.0, 32.8, 27.9%. The mean mass of 1 seed Nadwiślański variety from sowing to germination was 1.11 g and Tim variety 1.27 g. Dynamics of changing of the imbibing seeds mass treated or not by laser light was different (Fig. 5). Irradiated seeds were increasing of mass faster during bulking in comparison to seeds without irradiation.

Significant differences in dynamic of seeds germination both treated and not treated by laser light were also found (Table 1). Especially clear differentiation of germination dynamic counted as number of germinating seeds at particular times of harvest was observed after 24 h after sowing. Higher irradiation doses exerted a greater degree of influence on the number of germinating seeds than did lower doses. Significant differences in the dynamic of seeds germinating of both faba bean forms were found only from 48 to 96 h after sowing. All seeds sown achieved a 100% germinating capacity 144 h after sowing. Irradiation also has a moderate effect on the initial growth and development of faba bean seedlings, especially on length of the roots and coleoptile (Fig. 6). Higher doses of laser irradiation has more of an effect on the increment of plant organ length in comparison to lower doses.

For all used irradiation doses, the mean increment of coleoptile and root length from seedlings grown from irradiated seeds in relation to seedling grown from non-irradiated seeds was respectively: 32.6 and 28.4%. Tim variety seedlings had greater roots and coleoptile than that of the Nadwiślański variety.

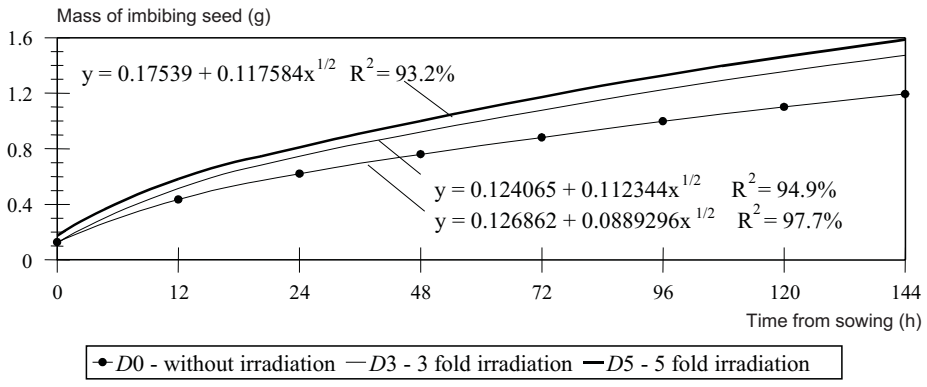

Fig. 5. Dynamics of change of seeds imbibing mass and faba bean seedlings treated and not-treated by laser irradiation.

T a b l e 1. Dynamic of germination of seed treated and not-treated by laser light expressed in (%) of germinating seed number

Description	Time from sowing (h)							
	0	12	24	48	72	96	120	144
Variety								
Nadwiślański	0.0 a*	2.6 a	12.5 a	22.4 a	38.5 a	42.7 a	49.2 a	50.0 a
Tim	0.0 a	3.7 a	12.8 a	18.6 b	33.6 b	50.2 b	49.7 a	50.0 a
Doses of irradiation								
*D*0	0.0 a	0.0 a	2.0 a	12.0 a	31.6 a	43.8 a	49.5 b	50.0 a
*D*1	0.0 a	3.9 b	13.6 b	18.5 b	36.0 b	48.5 b	49.0 b	50.0 a
*D*2	0.0 a	4.6 bc	13.8 b	20.5 b	44.5 c	49.0 b	49.0 b	50.0 a
*D*3	0.0 a	5.8 cd	14.0 bc	24.6 c	41.5 c	49.0 b	49.5 b	50.0 a
*D*4	0.0 a	5.5 d	15.5 c	23.3 c	40.5 c	49.5 b	50.0 b	50.0 a
*D*5	0.0 a	5.0 cd	14.0 bc	24.0 c	40.5 c	50.0 b	50.0 b	50.0 a

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

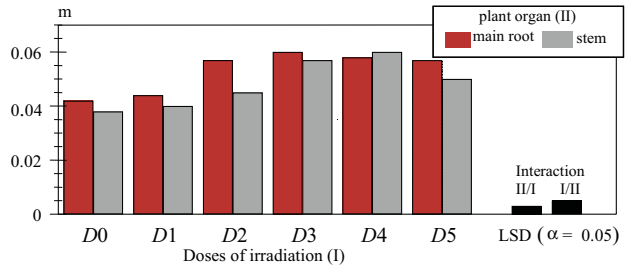

Fig. 6. Length of the faba bean seedling roots grown and the seedling stems grown from seeds treated and not-treated by laser irradiation.

DISCUSSION

The results of studies conducted showed a significant increase in the number of free radicals in those seeds which had been treated by laser irradiation at the pre-sowing stage. The concentration of free radicals in the seeds depended not only on the dose of irradiation but also on the faba bean form. In this paper, it has been shown that there is an optimal dose of laser irradiation with stimulatory effects on free radicals originating in the irradiated seeds. The calculated values of spectroscopy dispersions factor *g* for both faba bean varieties studied comprise from 2.0036 to 2.0042. Factor *g* for free electrons is 2.0023. On the basis of comparison these both factors values we can conclude that stable free radicals in the seeds are massive and they have surely less ability to move in comparison to free electron. It is in connection with this, that the *g* factors value in the faba bean seeds did not change before and after irradiation; we could suppose that free radicals contained in the seeds had a similar structure before and after irradiation.

We can find in bibliography that the large potency of irradiation often causes in the seeds an increasing number of free radicals to originate. In this situation can produce so-called active seat of radical which has an indirect effect on the causes of change and the breaking of their relative dormancy [6]. Earlier emergence, likewise faster rate of growth and development of plant which were grown from seed lot treated by physical factors, seemed to be among other things a consequence of these changes. Drozd *et al*. [3] were observed also the increment of free radical concentration after irradiation of winter wheat seeds by laser light. The size of these changes depended to a large degree on the dose of irradiation and wheat variety studied.

Faba bean seeds have been seen as a valuable fodder for animals for a long period of time. The increase of free radicals in the seed lot and seeds can considerably reduce the ability for it to be used as nourishment [15]. Detailed laboratory studies of seed lot harvested in the early phase of plant development which is the time when we can suppose the concentration of free radical is at its greatest did not show any extension in the number of free radicals either in those parts above ground or in the roots. Spectroscopy studies showed, that the concentration of free radicals in the seeds harvested from plants which were grown from the irradiated seeds lot was similar to the concentration of free radicals in the seeds without irradiation before sowing. Literature reports show, that the estimation of seed irradiation effect on changes in the plants which have been grown from these seeds is restricted most often only to a study of the chemical composition in the yield obtained [1,5,10]. It is hard to compare results which have been obtained in this experiment with results from other research because we were not able to find the papers concerning this problem in the Polish and foreign bibliography.

In our experiments, significant activation of both faba bean varieties emergence was found. Earlier emergence of plants as a result of faba bean seed lot irradiation was observed also in the experiments which were conducted under experimental field conditions [11]. The improvement of seeds germination which were treated, pre-sowing, by laser light but in referring to winter wheat was also found by Galova [4], Zhidong and Shuzhen [19].

The profitable effect of laser light on the increase of coleoptile and faba bean root length was found in the experiments carried out. It is evident that the changes shown earlier in the irradiation seeds were caused by them, too. In the opinion of the research workers busy with the problem of seed lot treating by laser light, the biggest changes occur in irradiated seeds and in the earlier period of the development of the plant from which it grew from these seeds [1,14]; these changes lead in the later period of development to faster plant growth. Irradiated faba bean seeds enlarged mass during imbibition in comparison to seeds without irradiation. It is caused probably by faster water absorption and therefore the dynamic of their emergence was greater. Investigations of Grzesiuk and Rejowski [7] showed, that maize seeds pre-sowing treated by ultrasound waves absorb decidedly more water than non treated seeds. Ionize radiation causes an activation of seed respiration and an increase of water absorbtion, too. Indeed ultrasounds and ionize radiation are physical factors so their affecting on seed lot of cultivating plants seems to be very similar.

CONCLUSIONS

1. A significant increase in the concentration of free radicals was found in the seeds pre-sowing irradiated by laser light. The biggest increase of free radicals numbers in the seed lot of both faba bean varieties was found after a 3-4 fold irradiation of seeds by laser light.

2. Irradiated seeds of both faba bean forms had a greater mass at the imbibing stage than seeds without irradiation. A consequence of this process was their earlier and steadier emergence.

3. Pre-sowing laser seed stimulation had a positive influence also on the growth and development of seedlings which were grown from these seeds. Faba bean seedlings which were grown from irradiated seeds had greater coleoptyle and lengther roots than control ones.

4. No significant effects were found of the irradiation of the seeds of the faba bean variety studied on free radicals concentration in roots and the above-ground part of plants. The number of free radicals did not differ significantly from that found in the same organs of plant grown from not irradiated seeds. Neither did spectroscopy research show any increased number of free radicals in the seeds harvested from plants which originated from irradiated seed lot.

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