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Effect of laser irradiation on the biochemical changes in seeds and the accumulation of dry matter in the faba bean

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A b s t r a c t. The experiment was conducted in the greenhouse of the Institute of Soil Science and Plant Cultivation in Puławy. The aim of the studies was the evaluation of some biochemical and physiological changes in faba bean (*Vicia faba minor*) seeds and the assessment of the dynamics of the accumulation of dry matter in plants grown from seed treated with laser light before sowing. Irradiation of faba bean seeds of the variety Nadwiślanski, significantly affected the activity of amylolitic enzymes in the seeds, especially in the germination initial period. Both the three- and five-fold treatment increased enzyme activity to the same degree. It was found that there was a significant effect of seed biostimulation on the scale and rate of dry matter accumulation of particular faba bean organs; the three-fold dose led to an increase in the dynamics in the above- ground part, whereas the five-fold dose, to that in the roots. The weight of vegetative organs intensively reached the phase of faba bean flowering; the highest increase of the total above-ground part of the weight was noted during flowering and pod setting, and was followed by a very fast increase in the weight of the generative organs. Irradiation of the seeds significantly influenced plant germination and modified the course of particular development stages of the faba bean resulting in the accelerated germination and maturity of the plants.

K e y w o r d s: faba bean, enzymes activity, laser light, biostimulation, yield

INTRODUCTION

The beneficial effect of the pre-sowing laser biostimulation of seeds on germination, initial development and yield has been already proved by numerous studies on some cereals [4,5], root crops [16] and vegetables [15]. Papers published by the author have provided the evidence that the development and yield of some legumes are also favourably affected by such treatment both under strictly controlled conditions [9] and in field experiments [7,8]. However, the influence of laser light has not been fully recognised and explained yet as there are only fragmentary studies or hypotheses that may just help to explain this impact [11,14]. Therefore there is a need to run a more thorough investigation focused on the biochemical and physiological processes taking place in treated seeds and plants.

The aim of the studies was to evaluate some biochemical and physiological changes in the seeds and give a detailed description of the dynamics of the accumulation of dry matter of the faba bean after application of the pre-sowing biostimulation of seeds with laser light.

MATERIALS AND METHODS

The experiments were carried out at the Department of Forage Crop Production of the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy, and laboratory analyses of seeds and seedlings at the IUNG Department of Biochemistry and Crop Quality. Seeds were irradiated at the Department of Physics of the University of Agriculture in Lublin using equipment for the laser biostimulation of pre-sowing seeds [6].

The experiment was based on the faba bean (*Vicia faba minor*) of the variety Nadwiślański and the following doses of laser light: D_0 – no irradiation, D_1 – single irradiation, D_2 – double irradiation, D_3 – three-fold irradiation, D_4 – four-fold irradiation, D_5 – five-fold irradiation. A single exposition was equal to $4 \cdot 10^{-3}$ J·cm^{-2}·s^{-1}.

In order to determine the activity of amylolytic enzymes, the seeds were irradiated, sown in Petri dishes, and then analysed after 12, 24, 48, 72, 96, 120 and 144 h. The amount of glucose released from the starch (I) by the complex of

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enzymes contained in the supernatant was assumed as a measure of enzyme activity. Supernatant was obtained by adding 5 ml acetate buffer (pH 4.8) which contained 20 μmoles $CaCl_2$ to 0.5 g of ground, dried plant material and rotation during 10 min at 3000 turns per min. To 0.5 ml of supernatant was added 0.5% of starch and realized hydrolysis at temperature 370°C during 10 min. The modified Somogy-Nelson method [12], one of the most frequently used in biochemical experiments to determine the content of reducing sugars, was applied to assess the content of glucose. In this method was used colour reaction of arsenic-molybdenum reagent with cuprous ions, which origin in solution as a result of reaction of copper reagent with reducing sugars. Intensity of colour was colorimetric measured at wave length λ = 520 nm. Concentration of reducing sugars was stated on the base of calibration curve which was made for glucose solution.

The studies aimed to determine the dynamics of faba bean yield increase were run in Mitserlich pots at the Vegetation Hall of the Institute of Soil Science and Plant Cultivation in Puławy. The experiment was based on the faba bean of the variety Nadwiślański and the following doses of laser light: D_0 – no irradiation, D_3 – three-fold irradiation, D_5 – five-fold irradiation. A single exposition was equal to $4 \cdot 10^{-3}$ J·cm^{-2}·s^{-1}.

Detailed observations of plant growth and development were recorded throughout the whole vegetation period. In order to evaluate the dynamics of fresh and dry matter increase, plants were harvested 7 times ($T1$ – seedling stage, $T2$ – 2–3 leaf stage, $T3$ – 5–6 leaf stage, $T4$ – flowering of main shoot, $T5$ – pod setting on main shoot, $T6$ – full seed filling on main shoot, $T7$ – full maturity of seeds on main shoot and lateral shoots), which had been preceded by noting the occurrence of the more important development stages and characterising plants. At the harvest the following parameters were evaluated: height to the first and to the last pod and to the top of the plant, leaf area, number of leaves, fresh and dry matter of particular parts, and also, at later harvest times-number of pods, average pod length, weight of stripped pods, number and weight of seeds, seed humidity. The results of the studies calculated as mean values from 3 pots were processed statistically by the analysis of variation, based on the Tukey semi-interval confidence at the significance level α = 0.05.

RESULTS AND DISCUSSION

The activity of amylolitic enzymes was found to increase in time, reaching its highest value between 24 and 72 h after sowing. Irradiation significantly changed enzyme activity in the seeds, particularly at the initial stage of their germination (Fig. 1) but both the three- and the five-fold treatment proved to give similar results. Biostimulation of faba bean seeds led to a rise in the activity of amylolitic enzymes for three- and five-fold doses after 12, 24, 48, 72, 96, 120 and 144 h after sowing by: 18.1, 77.4, 114.0, 90.2, 40.0, 16.6, 21.2%, respectively. The changes of the enzymatic activity of some winter wheat varieties were also observed by Galova [2] after irradiation of kernels by He-Ne laser light. The dynamics of germination, as indicated by the number of seeds that germinate particularly at harvest time, significantly differed between treated and untreated material (Fig. 2), being particularly distinct after 12 and 24 h after sowing.

Higher doses of irradiation increased the number of seeds, which had germinated to a greater extent than the lower ones. 144 h after sowing, all the seeds reached germination capacity equal to 100%. The biostimulation of

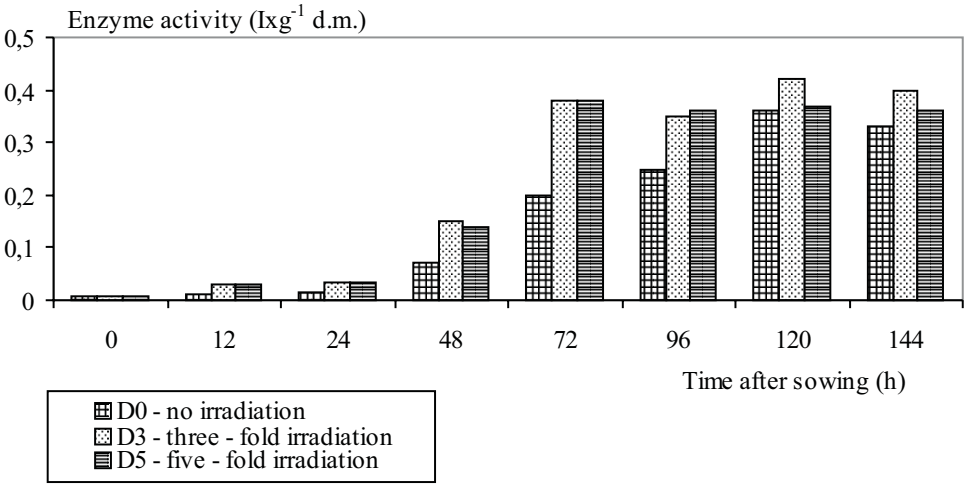

Fig. 1. Activity of amylolitic enzymes in faba bean seeds and seedlings treated and untreated with laser light.

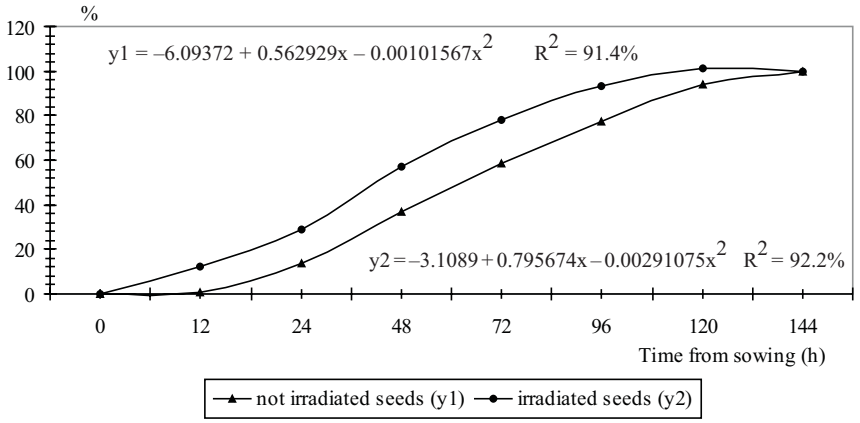

Fig. 2. Dynamics of treated and untreated seed germination.

seeds positively affected the initial growth and development stage of faba bean seedlings, in particular the length of the root and coleoptile. The increased germination capacity as a result of irradiation of spring wheat seeds by laser light was observed earlier by Drozd and Szajsner [1] and Zhindong and Shuzhen [17].

Previous research [10] also showed significant differences in concentration of free radicals as noted in the samples of both treated and untreated seeds. The number of free radicals increased at first as the dose of irradiation increased, and then, after reaching the maximum, decreased. However, it was found that the pre-sowing treatment had no significant influence on the concentration of free radicals in the part below the ground and in the roots of the faba bean plants grown from both treated and untreated seeds neither did it affect the concentration in seeds gathered from these two species of plant. The average number of free radicals found in treated and untreated faba bean seeds was $1.7 \cdot 10^{14}$ spins g^{-1} in the above-ground part and $3.1 \cdot 10^{14}$ spins g^{-1} in roots.

Faba bean seeds grown in pots started to emerge evenly, 17 days after sowing. Irradiation of the seeds influenced the rate of the occurrence of the first plants, but the plants grown from three- and five-fold irradiated seeds did not differ significantly with regard to the time and evenness of germination. Seeds treated with laser light germinated about 2–3 days earlier than did the untreated ones. Differences were observed in growth and development between plants grown from treated and untreated material; these were particularly distinct during initial plant growth, from the 2–3 leaf stage to the beginning of flowering. Plants obtained from treated seeds were higher and began to flower about 3–4 days earlier when compared to the plants from untreated seeds. The first nodules were present on the roots of faba bean seedlings 12–14 days after germination – the irradiation of seeds had not influenced the date of the formation of the first root nodules.

The highest increase of dry matter yield for particular parts of faba bean occurred during budding and flowering (Fig. 3). In further development stages, as a result of drying, the great decrease in the yield of dry matter of the roots and the slight decrease in the yield of both stem and leaves were recorded, but the yield of seeds and stripped pods considerably increased. After flowering, the increase of dry matter yield was found to be significantly lower than at the initial development period. The dry matter yield of the whole aboveground part of the plants rose within their growth and development up to the stage of seed filling, and the roots – until the stage of bud forming. The weight of the vegetative organs of the faba bean reached its highest increase in the period preceding flowering and reached the maximum at bud forming on all objects. After that time the weight of vegetative organs proportionally decreased, while the generative organs increased their weight very quickly.

The pre-sowing biostimulation of seeds essentially affected the rate of dry matter accumulation of particular plant parts. The highest influence of the treatment on the yield of dry matter was observed during germination and from the 5–6-leaf stage until flowering, thus at the time when plants were growing intensively. Afterwards irradiation was found to influence mostly the yield of stripped pods and seeds. The increase in the yield of the dry matter of the seeds was similar after both the two- and the five-fold treatment. At the bud forming stage the increase of dry matter yield of the roots, leaves and stems after three-fold irradiation was: 20.8, 43.4, and 31.2%, respectively, whereas a five-fold irradiation caused an increase of about: 36.2, 41.6, and 44.1%, respectively. Laser light influenced the increase in the dry matter yield of particular plant parts to a similar extent.

Detailed biometric measures of plants carried out before the harvest proved that irradiation of seeds modified some morphological features of the faba bean. The leaf area of plants grown from treated material increased more quickly

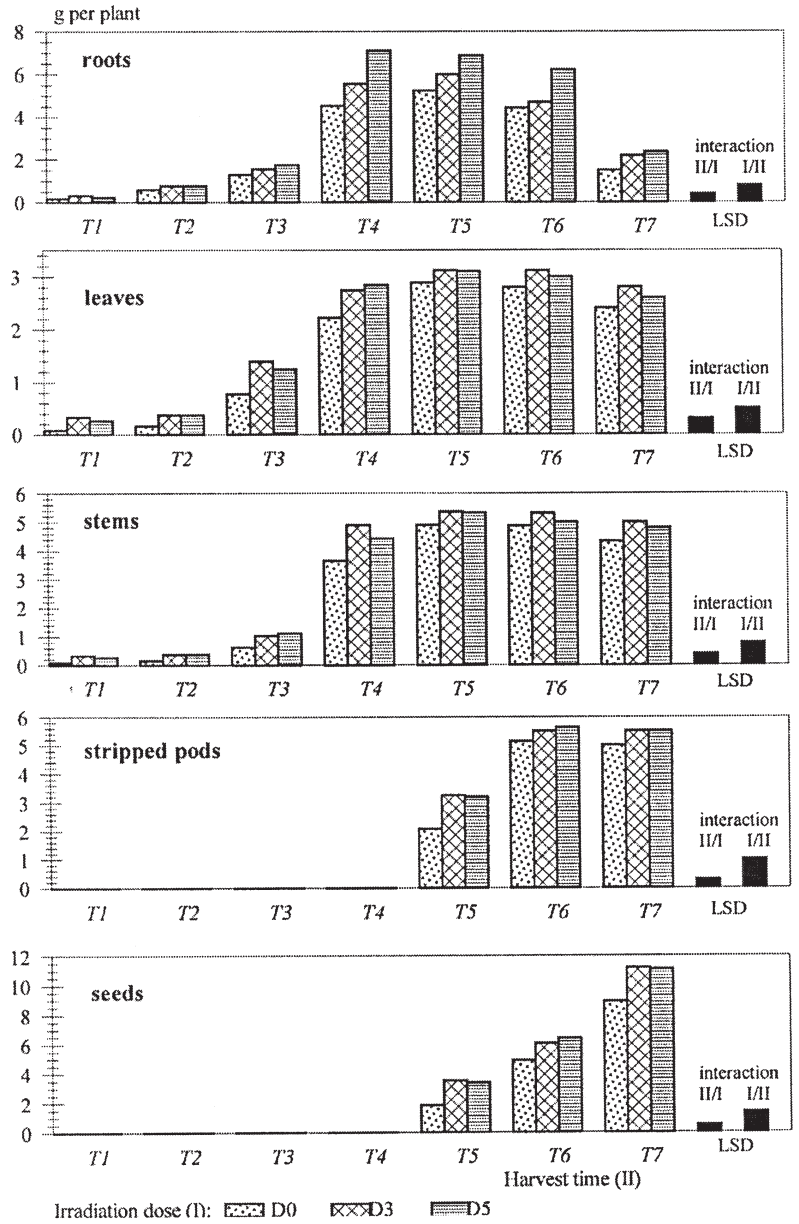

Fig. 3. Dry matter yield faba bean plants grown from seeds treated and untreated with laser light.

Table 1. Value of some morphological and functional features of pre-harvested faba bean plants grown from seeds treated and untreated with laser light

Specification	Plant height (cm)	Length of stem with pods (cm)	Number of pods per plant	Number of seeds per plant	1000 seed weight (g)	Mean length of pod (mm)
Irradiation doses:						
D_0	89.2a	2.5a	9.8a	2.6a	558a	64.4a
D_3	93.4b	26.9b	11.3b	2.6a	584b	69.7b
D_5	91.3c	27.2b	11.5b	2.7a	592b	68.9b

Values in columns followed by the same letters do not differ significantly.

during the period between germination and flowering and then decreased faster in time when compared to plants grown from untreated material. Pod density was found greater in the case of plants grown from treated seeds (Table 1), resulting in elongated pod stems. The seeds of plants grown from treated material were characterised by a higher per 1000 seed weight, which was probably an effect of the increased length of the pods, because the number of seeds per pod did not significantly change. Plant height was modified to a much lower extent.

Detailed analysis on the content of dry matter in particular parts proved that before the harvest, plants grown from treated seeds contained 8.6, 34.2, 11.0, 12.6% water in leaves, stems, roots and seeds, respectively, and from untreated material respectively: 14.1, 41.4, 11.2, 17.4%, indicating the quicker loss of water or earlier maturity of plants grown from the treated irradiated seeds.

This fact has been already explained by Grzesiuk and Kulka [3], according to whom some physical factors may affect crop seeds and may accelerate the maturity of plants grown from such kind of material.

CONCLUSIONS

1. Irradiation of seeds of the faba bean variety Nadwiślański significantly changed the activity of amylolitic enzymes, particularly in the initial stages of germination. Both the three- and five-fold treatment of seeds were the most favourable but had a similar influence on the increase of enzyme activity.

2. It was observed that seed irradiation significantly affected the rate and the amount of dry matter accumulation of particular parts of a plant. The three-fold irradiation of seeds was found to be more beneficial for the dynamics of the increase of the aboveground part and five-fold irradiation – of roots.

3. The rate of accumulating the weight of vegetative organs increased intensively until flowering; the highest increase in the weight of the whole aboveground part was recorded during flowering and pod formation. After that time the weight of the vegetative organs decreased while the mass of generative organs of the faba bean – increased very quickly.

4. Pre-sowing seed biostimulation positively affected plant germination and modified the course of particular development stages, which led to accelerated germination and maturity as a result of the lower content of water in the seeds.

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