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Impact of presowing laser irradiation of seeds on sugar beet properties

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A b s t r a c t. The aim of the experiment was to establish the influence of biostimulation on the sugar beet seeds. The seeds came from the specialized breeding program energ'hill or were irradiated by the laser in two doses. The impact of the biostimulation was analyzed by determining the nitrate reductase activity and the nitrate, chlorophyll and carotenoids contents in leaves, as well as, the dry matter and sugar concentration in mature roots. The field experiment was established for two sugar beet cultivars. Biostimulation by irradiation and a special seed breeding program energ'hill had a positive influence on some examined parameters (particularly on nitrate reductase activity in Ruveta and in numerous cases on photosynthetic pigments in both cultivars). Regarding the dry matter accumulation and sugar concentration this impact was more favourable for Tiziana than for Ruveta cultivar.

K e y w o r d s: seed biostimulation, laser radiation, nitrate reductase, chlorophyll, sugar concentration

INTRODUCTION

Current agronomical research is directed towards the elaboration of effective methods of the plant physiological activity improvement and crop yield. One of these methods is laser pretreatment of crops seeds. The literature data indicate that laser irradiation increases seed germination and improves plant growth and metabolism (Chen *et al.*, 2005a, b; Drozd and Szajsner, 2007; Perveen *et al.*, 2010; Podleśny 2002). Numerous studies have proved that laser irradiation improves plant resistance to some environmental stresses (Chen, 2008; Qi *et al.*, 2000; Qiu *et al.*, 2008). The influence of laser irradiation on plants is most likely connected with its light and electromagnetic effects. Plant growth and development is controlled by internal and external factors with red and far-red light (and their receptors – phytochromes) playing a very important role. Chen *et al*. (2005b) holds that laser irradiation might activate phytochrome which consequently modulates plant response. On the other hand, excess of energy absorbed by seeds, may accelerate plant metabolism and finally result in its growth improvement. One of the positive effects of laser biostimulation is the increased concentration of photosynthetic pigments in leaves. Plant growth and productivity is dependent, among others, on nitrogen availability and its assimilation. In moderate climate regions the main source of nitrogen for many plant species is nitrate. A high amount of nitrate is allocated to the leaves where it is assimilated or stored in vacuoles. Nitrate reductase (NR), an enzyme that reduces NO_3^- to NO_2^-, plays a central role in nitrogen assimilation and is considered as a limiting step in this pathway.

The main objectives of this study were to examine the effect of presowing laser irradiation of seeds on:

– some physiological parameters (NR activity, nitrate content and concentration of phosynthetic pigments) in leaves of sugar beet at different stages of vegetative growth;
– dry matter accumulation and soluble sugar content in roots.

Secondary goal was to compare the obtained results for plants emerged from laser biostimulated seeds with the results obtained for cultivars selected in specialized seed breeding program (energ'hill).

MATERIALS AND METHODS

The effect of seed material biostimulation of two diploid cultivars of sugar beet Ruveta (sugar type) and Tiziana (sugar – normal type) from Syngenta company has been

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analyzed by comparing the seed subjected to a specialized seed breeding program (energ'hill) and laser irradiation to non-treated seeds. The most important part of energ'hill technology is the tightly controlled imbibition of seeds in water (the germination processes are initiated) and then quick drying (germination is stopped).

The presowing irradiation was conducted with the use of semiconductor laser light (CTL – 1106 MX) with the power of 200 mW and a wavelength of 670 nm. The irradiated surface was set with the help of a scanner (CTL 1202 S) cooperating with the laser. The 5-time multiplied (D5) and 7-time multiplied (D7) of the basic dose (0.25 J cm^{-2}) as well as the controlling combination (without irradiation) have been applied.

The seed material was sown immediately after the irradiation. The field experiment was conducted in the climatic conditions of Lower Silesia on the brown earth soil of fluvioglacial origin (Griffon type), medium clay of good wheat complex (pH 6.1-6.5) and rich in minerals. Two-factorial experiment was established on April 19th, 2009, according to the method of split plot in three replications.

Air temperatures in 2009 were favourable to vegetation and compared to the average temperatures of the last 30 years varied in the range of 1.0-2.5°C. The temperature changes did not have an evident effect on plant growth. Precipitation distribution, however, was not uniform. In June and July it amounted 141.7 and 134.2 mm, respectively and from the third decade of August and in September periodic drought (5.7 and 12 mm) was observed.

In the first half of July, August, September and October (3.07, 11.08, 8.09, 14.10.2009) the samples from all plots were collected and the experiment was finished on October 22, 2009.

The plants were analyzed from early stages of vegetative growth to the production of storage organs (four times during vegetation period). They were collected between 8 and 9 a.m. and immediately transported to the laboratory for examination. The following three parameters were examined: the activity of nitrate reductase (NR), the nitrate content and the concentration of photosynthetic pigments. All these parameters were analyzed in the youngest fully expanded leaf. All samples were taken from the middle part of a leaf, without main midribs. Nitrate reductase activity was determined according to the Jaworski method (1971). Plant material (0.5 g) was cut into 5 mm segments and placed in test-vial containing 20 cm^3 phosphate buffer (0.1 mol dm^{-3} K_2HPO_4-KH_2PO_4, contained 1% isopropanol pH 7.5). The test-vials were vacuum infiltrated for 2 min and then incubated in the dark at 25°C for 1 h. The amount of nitrite formed during this reaction was measured spectrophotometrically at 540 nm after adding 1% sulphanilamide in 100 mmol dm^{-3} HCl and 0.01% N-naphtyl-ethylenediamine. To determine nitrate content, 1g of fresh plant material was extracted with distilled water at 100°C for 10 min. After filtration through Whatman No. 1 filter paper the nitrate was determined colorimetrically according to the method of Cataldo *et al*. (1975). The samples were mixed with 5% (w/v) salicylic acid in concentrated H_2SO_4 and incubated for 20 min at ambient temperature. The colour developed after the addition of 2 mol dm^{-3} NaOH and the absorbance at 410 was measured after the mixture was cooled to room temperature. To extract photosynthetic pigments 80% acetone was used and to calculate the concentrations of chlorophylls and carotenoids the Lichtenthaler equations (1987) were applied. Results of all biochemical analyses are the mean of six determinations.

In mature roots from all field experiments repetitions the amount of dry matter was determined by drying in the temperature of 105°C and the amount of sugar was determined by the use of refractometric method.

The data for all parameters were statistically analyzed by the analysis of variance. Differences among mean values of all treatments (control, D5, D7, energ'hill) were compared by LSD test ($P<0.05$).

RESULTS AND DISCUSSION

Nitrate reductase activity (NRA) in leaves of non-biostimulated Ruveta plants remained relatively stable during the entire experimental period (Fig. 1a). Plants emerged from seeds treated by D5 laser dose had markedly lower NRA at early stages of growth (75 and 114 days after sowing – DAS) and then this activity increased significantly (approximately by 30% in comparison to control plants). On the other hand NRA in plants emerged from seeds treated by D7 laser dose was significantly higher than in control plants during the examined period and it was very similar to the activity determined in plants raised from energ'hill seeds. In this case, the highest increase in NRA was observed at 75 DAS and it accounted 66% compared to control plants. At the early stages of growth (75 and 114 DAS) the nitrate concentration in control plants was significantly higher than in the remaining variants of experiment. At 178 DAS plants biostimulated by D5 laser dose contained the highest amount of nitrate (15.08 μmol $(NO_3^-)g^{-1}$ FW) and it was by 20% higher as compared to control plants (Fig. 1b).

In Tiziana cultivar NRA was very changeable and the peak of activity was observed at 142 DAS (for all variants). Similarly to Ruveta cultivar, D5 laser dose caused significant decrease in NRA at early phases of growth and marked increase at 178 DAS. Contrary to Ruveta, in plants emerged from seeds treated by higher laser dose NRA did not differ significantly from the control plant (with the exception of final analysis). During the vegetation season NRA in plants emerged from energ'hill seeds was also highly diversified but it was higher than in control plant only in the first phase of growth (75 DAS). Plants emerged from seeds treated by D5 laser dose contained less nitrate in comparison to remaining treatments (with the exception of final determination). The nitrate concentration in plants emerged from energ'hill

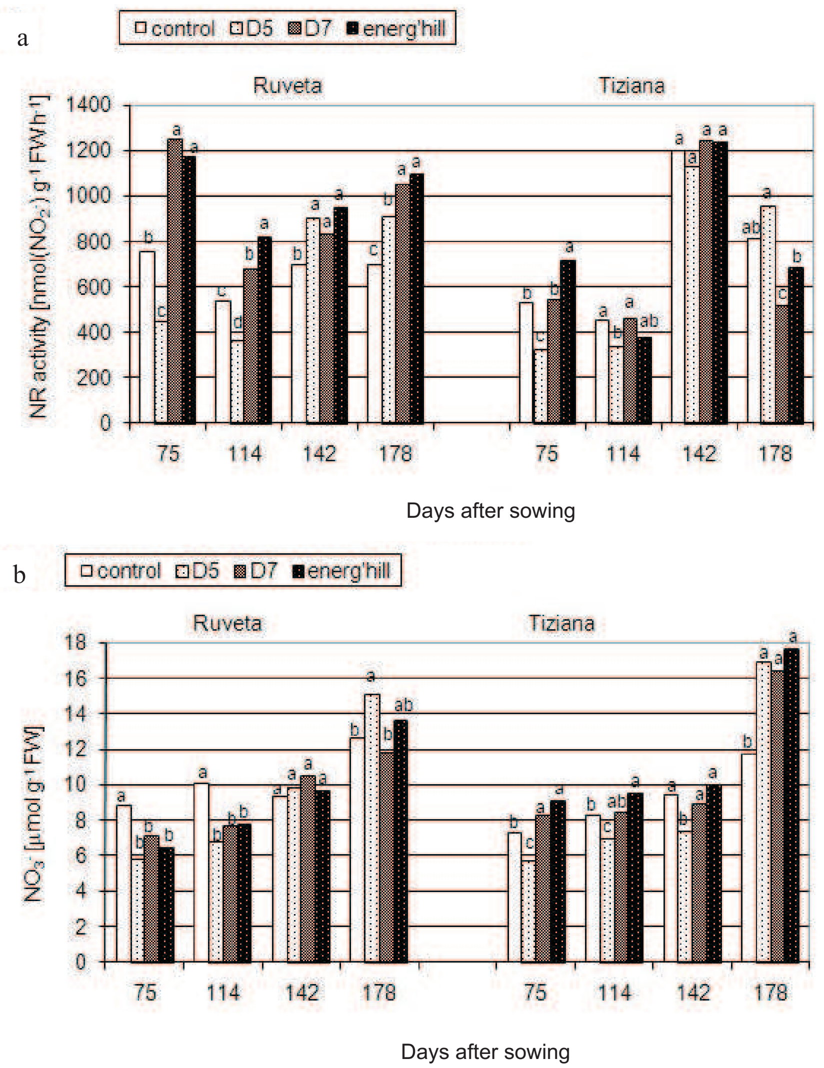

Fig. 1. Influence of laser pretreatment of seeds on: a – the nitrate reductase activity, b – the nitrate content in leaves of two sugar beet cultivars (Ruveta, Tiziana) at different harvest dates. Mean values at the same harvest date with the same letter above the bars do not differ significantly at $P<0.05$.

seeds was relatively stable but noticeably higher than in control plant (Fig. 1b). At the terminal phase of growth (178 DAS) there was a strong increase in nitrate concentration (80% as compared to earlier phases). It is worth noting that both examined cultivars showed a tendency to accumulation of nitrate at the final stage of growth.

The total concentration of chlorophyll (chl a + b) in the leaves of Ruveta pre-treated by laser was generally higher than in non-biostimulated plants (Fig. 2a). However, the statistically significant increase was observed only at the first phase of growth (75 DAS). In the case of plants emerged from energ'hill seeds there was approx. 10% increase in the total chlorophyll content in comparison to control plants. This positive effect sustained during the whole growing season. Similar trend was observed in carotenoid concentrations in the plants emerged from energ'hill seeds and in plants raised from seeds treated by D5 laser dose (Fig. 2b). Higher laser dose caused the increase in carotenoid concentration only at the initial stage of growth (75 DAS).

In Tiziana cultivar, plants raised from seeds treated by D5 laser dose contained more chlorophyll than non-biostimulated plants and those stimulated by a higher dose of laser. Similar tendency was observed in carotenoids concentration. The leaves of plants emerged from energ'hill seeds had the increased level of total chlorophyll in comparison to control plants. However, statistically significant differences took place at the later phases of growth (142 and 178 DAS).

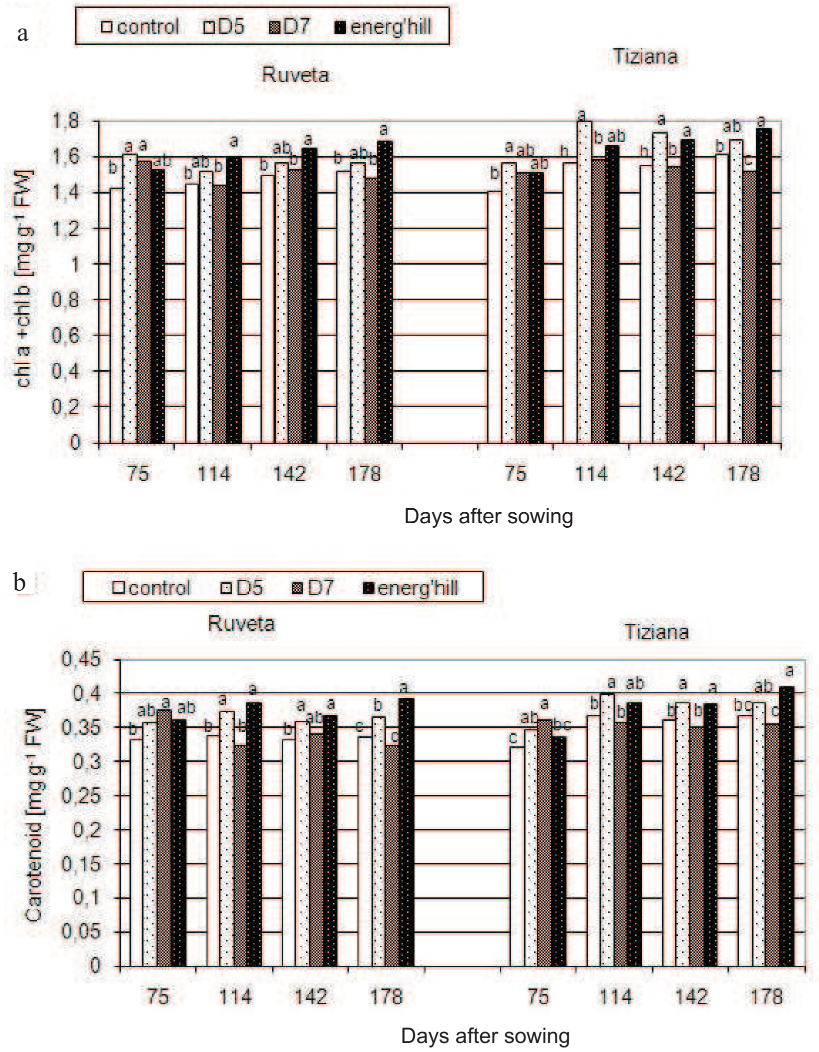

Fig. 2. Influence of laser pretreatment of seeds on: a – total chlorophyll, b – carotenoids concentration in leaves of two sugar beet cultivars (Ruveta, Tiziana) at different harvest dates. Explanations as on Fig. 1.

Specialized seed breeding program (energ'hill) and the laser biostimulation of seeds altered the concentration of dry matter and sugar content in the root (Table 1). The impact of these factors was different for the analyzed cultivars. In both cultivars the most effective for the dry matter and sugar content was the laser irradiation at a 7 time multiplied (D7). In Tiziana cultivar, both laser doses caused significant increase in dry matter and sugar content compared to control. Plants of Tiziana cultivar raised from energ'hill seeds showed higher dry matter accumulation and sugar content in comparison to control.

Researches on the effect of laser biostimulation on plants mainly concern seed germination, plant emergence, yield quantity and in rarer cases yield quality (Gładyszewska, 2011;

T a b l e 1. Content of dry matter and sugar in roots of plants raised from seeds treated by laser and selected in breeding program (energ'hill)

Treatment	Ruveta		Tiziana	
	Dry matter (%)	Sugar (%)	Dry matter (%)	Sugar (%)
Control	22.7 b	16.14 b	22.7 d	15.71 c
Laser D5	22.5 c	16.05 b	23.3 b	16.30 ab
Laser D7	23.8 a	16.35 a	24.0 a	16.43 a
Energ'hill	22.7 b	16.08 b	23.1 c	16.13 b
$LSD_{0.05}$	0.20	0.20	0.20	0.20

Ivanova, 1999; Muszyński and Gładyszewska, 2008; Osman *et al*., 2009; Perveen *et al*., 2010). Examinations usually are limited to one harvest at the end of the vegetation period (Koper *et al*., 1996, Rochalska *et al*., 2008, Rybiński and Garczyński, 2004). Our examinations were conducted at different developmental stages of the plants and we wanted to establish whether there are differences in some physiological parameters in plants emerged from seeds stimulated by laser irradiation or non-stimulated. For the assessment of plant vigour we chose three parameters: the nitrate content, the nitrate reductase activity and the photosynthetic pigments concentration. Sugar beet is primarily cultivated for high yield of dry root matter and sucrose. Nitrogen is a crucial nutrient for optimizing its growth and yield and hence nitrate plays an important role in its growth (Raab and Terry 1994). On the other hand, nitrate is an important signalling molecule that triggers expression of nitrate-specific genes *eg* expression of nitrate reductase genes. Nitrate reductase (NR) – the key enzyme in the first step of nitrate assimilation pathway – determines the rate of nitrogen assimilation in higher plants (Campbell, 1999; Reed and Hageman, 1980) and it is considered as one of the important elements controlling nitrogen use efficiency (NUE). A large portion of nitrogen is transported to the leaves throughout the whole life of plants and the considerable part of nitrogen is invested in photosynthetic apparatus.

Literature data claim that laser radiation has a positive effect on plant growth and metabolism (Chen, 2008; Chen *et al*., 2005b; Podleśny, 2002; Rybiński and Garczyński, 2004) but information on nitrate assimilation is not available. Our experiments showed that 75 and 114 DAS, plants emerged from seeds treated by D5 laser dose had significantly lower NRA than non-stimulated plants. On the other hand higher laser dose caused significant increase in NRA in leaves of Ruveta and did not change activity in Tiziana. In later phases of growth there was observed increase in NRA in Ruveta plants emerged from seeds treated by both doses of laser irradiation and plants from energ'hill seeds in comparison to the control plants. It can be assumed that these changes in NRA were related with synthesis rate of NR protein. It is worth to noting that NRA sustained at relatively high level during the whole experimental period including the end of the growing season. High NRA at the end of vegetative season might be connected with the enhanced nitrate concentration in leaves (Fig. 1). This increased level of nitrate might result from allocation of ions and metabolites within a plant during its maturation (increased sucrose export from shoots to roots and nitrate from roots to shoots). Scheible *et al.* (1997) maintain that NO_3^- accumulation in leaves inhibits starch synthesis and it results in the increased sucrose level. Our findings indicated that in the case of Ruveta cultivar the highest sugar concentration was in plants raised from seeds treated by D7 laser dose but the difference was relatively small compared to remaining treatments. In Tiziana cultivar both laser doses caused a slight increase in sugar content comparing with the control. Koper *et al*. (1996) examined four varieties of sugar beet and four doses of laser radiation and they did not state the enhancement of sugar content. Nevertheless, they showed that root biomass and biological yield of sugar in some cases were significantly improved by laser biostimulation. The results of our investigation showed that roots of Tiziana reacts positively on laser biostimulation. Moreover, we noted two interesting features of this cultivar:

– relatively high activity of nitrate reductase at two final harvests (142 and 178 DAS) in comparison to earlier phases, and
– high concentration of total chlorophyll during the whole growing season (particularly in the plants emerged from both energ'hill seeds and seeds treated by laser D5).

Djennane *et al*. (2004) stated that the enhancement of the nitrate reduction rate may lead to higher biomass production. Chen et al. (2005b) showed that laser pretreatment induces statistically significant changes in some biochemical and physiological parameters in seedlings of *Isatis indogotica*. Laser treatment of seeds resulted in 44% increase in total chlorophyll concentration and 31% increase in the content of soluble sugar compared to non-biostimulated plants. In our experiments the greatest increase in the total chlorophyll content reached 15% in comparison to the control and it was observed at 114 DAS in Tiziana plants emerged from seeds treated by D5 laser dose. Experiments conducted by Rochalska *et al*. (2008) on sugar beet showed that chlorophyll content and plant yield may be stimulated by presowing treatment of seeds by a low frequency magnetic field. On the other hand, Hernandez-Aguilar *et al*. (2009) showed that the presowing laser stimulation of seeds may negatively effect the concentration of chlorophyll a in maize leaves. Moreover, they found that the effect of laser irradiation was dependent on the seed genotype.

The positive influence of seeds biostimulation that resulted in the yield growth and the sugar content was stated by Wójcik and Bojarska (1998). Similarly, Podleśny (2002) also observed a positive effect from laser biostimulation on some physiological and morphological parameters of faba bean plants.

Thus, our experiments showed that the examined biochemical parameters (NRA, NO_3^- content and chlorophyll concentration) were very changeable during the growing season and there were not clear and obvious relationships between laser pretreatment and analyzed parameters. It can be assumed that the effects of laser stimulation of seeds reveal at all developmental stages of plants but they are especially evident at the early phase of growth.

CONCLUSIONS

1. Tiziana cultivar more positively responded to laser stimulation than Rouveta and there were slight increases in root dry matter accumulation and sugar concentration.

2. The special seed breeding program (energ'hill) may be an efficient method for improving plant productivity.

3. The obtained results indicate that the presowing laser irradiations of seeds may modify physiological processes at later developmental stages of plants.

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