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EFFECT OF LASER- AND HYDROPRIMING OF SEEDS ON SOME PHYSIOLOGICAL PARAMETERS IN SUGAR BEET

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Abstract

The aim of this study was to evaluate the effect of pre-sowing seed treatments on some agro-physiological parameters of sugar beets. The selected seed treatments were laser stimulation, hydropriming and a combination of hydropriming and laser irradiation. The impact of the stimulation of seeds was analysed by determining the acid phosphatase activity, concentration of phosphate and photosynthetic pigments in leaves as well as the nutrient status and some yield parameters of the roots. The plants were assayed four times during the growing period. The experiment showed that the priming methods had a relatively small effect on both the level of orthophosphate and total phosphorus concentration. On the other hand, the plants raised from seeds both hydro- and laser-primed preserved a more stable level of phosphates during the whole growing season than the other plants. The highest activity of acid phosphatase was observed in younger plants and among different seed treatment, plants grown from non-primed seeds and laser-primed seeds showed the highest enzyme activity. In later growth stages, enzyme activity lowered considerably. Considering the effect of different seed treatments, a significant increase in enzyme activity was observed in plants emerged from hydroprimed or laser stimulated seeds. In addition, an increase in chlorophylls occurred in plants emerged from hydroprimed seeds and from hydro- and laser-primed ones. Seed stimulation had a positive effect on the potassium content, particularly in leaves of sugar beet, and on some yield parameters of the roots. Concluding, the effect of pre-sowing seed stimulation (priming) was visible during the entire growing season. Out of the examined seed treatment methods, hydropriming alone or in combination with laser radiation caused the highest alterations in the assayed parameters.

Keywords: priming, laser stimulation, phosphorus, acid phosphatase, chlorophyll, carotenoids, sugar, nutrient status.

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INTRODUCTION

A challenge that modern agriculture faces is to provide the right amount of high-quality food. There are several barriers to achieving this aim, due to the growing shortage of agricultural areas, climate change, environmental pollution and other biotic and abiotic stresses (water shortage, inadequate temperatures, UV radiation, plant diseases, etc.). Therefore, agricultural scientists try to find effective methods for improving the crop production under both favourable and stressful conditions. This aim may be achieved through an application of non-nutritional additives supplied to the root zone or directly to leaves by spraying (Sacała, Durbajło 2012, Artyszak et al. 2014). Another solution is to submit seeds before sowing to certain treatments that may improve their vitality and result in better crop yield. An important approach to pre-sowing seed treatment is priming (Finch-Savage et al. 2004, Ashraf, Foolad 2005, Chen, Arora 2013, Ouhibi et al. 2014). According to Conrath (2011), priming enables cells to respond to very low levels of a stimulus in a more rapid and robust manner than non-primed cells do. In plants, priming plays a role in defense ('defense priming') and seed germination ('seed priming'). Different chemical and physical factors have been used as priming stimuli. Among the latter, pre-sowing treatment of seeds by laser irradiation (laser biostimulation) seems to be a very promising method. Another popular technique is hydropriming, which involves imbibition of seeds to a point where germination processes are initiated but not completed. Treated seeds are usually redried before using and after rehydration they demonstrate rapid germination and stand establishment (Ashraf, Foolad 2005). Literature data show that both of these priming methods (laser stimulation and hydropriming) can effectively enhance the germination rate and improve the vigour of seedlings, plant growth and total fresh and dry mass (Wójcik et al. 2004, Hernandez et al. 2010, Sacała et al. 2012, Prośba-Białczyk et al. 2013). Priming effects can continue through later stages of development and in some cases they intensify in more advanced stages of the plant growth (Iqbal, Ashraf 2007).

In our previous experiments, we examined the response of sugar beet to two priming methods: (1) laser biostimulation at two doses of laser irradiation and (2) hydropriming by using primed seeds (energ'hill) obtained from Syngenta Company (Sacała et al. 2012, Prośba-Białczyk et al. 2013). The results of these experiments indicated that both laser biostimulation and special treatment of seeds (energ'hill) might successfully improve the physiological activity and productivity of sugar beet, which encouraged us to undertake the current study. However, we extended the scope of our research by adding a treatment with laser light in combination with hydropriming. The focus is on the influence of three different pre-sowing seed treatments (laser irradiation, hydropriming and a combination of hydropriming and laser irradiation) on some aspects of the phosphorus metabolism and nutrition-

al status in sugar beet. The laser dose to be tested was selected based on the results of our previous experiments (Sacała et al. 2012, Prośba-Białczyk et al. 2013).

MATERIAL AND METHODS

Plant material and condition of cultivation

Experiments were conducted on the sugar beet cultivar Tiziana. The pre-sowing seed treatments were as follows: (1) non-primed seeds as a control, (2) laser primed seeds (LP), (3) hydroprimed seeds (HP) and (4) a combination of hydropriming and laser irradiation (HP+LP). Laser irradiation was conducted with a semiconductor laser light (CTL – 1106 MX) with the power of 200 mW and a wavelength of 670 nm. The irradiated surface was identified with a scanner (CTL 1202 S) coupled with the laser. Seeds were exposed to a light dose that was the sevenfold of the basic dose (2.5 10^{-1} J cm^{-2}). Hydroprimed seeds were obtained from Syngenta Company. The seed material was sown immediately after the irradiation.

The field experiment was carried out at the experimental station of the Wrocław University of Environmental and Life Sciences in Pawłowice, on soils classified as Haplic Luvisols (FAO WRB, 2007) developed from sandy loam lying on sandy clay loam (soil texture defined according to the Polish classification of soil grain size distribution, PTG, 2008, based on USDA). The soil was slightly acidic (pH 5.9-6.4) and characterized by an average content of potassium (150-170 mg K_2O kg^{-1} soil) and phosphorus (130-170 mg P_2O_5 kg^{-1} soil). Fertilizers were used in the autumn in the amount of 160 kg K_2O ha^{-1} and 90 kg P_2O_5 ha^{-1}.

The experiment was established on 22th April 2010, according to the method of split plots in three replications, and the standard recommended agronomic practices were carried out throughout the growing season.

The sugar beet growth proceeded under thermal conditions that did not impede the plants' development. Mean temperatures in 2010 were similar to temperatures of the last 30 years. The distribution of precipitations was favourable to the cultivation of sugar beet plants. From June to the end of September, the following precipitation amounts were recorded: 36.6 mm in June, 65.6 mm in July, 94.0 mm in August and 27.9 mm in September. The average total rainfall in the whole period reached 224.1 mm. More details can be seen in Figure1.

The plants were analysed four times during the period. Samples from all the plots were collected in the first decade of July, August, September and October, and the experiment was finished on 25th October. Three plants from each plot were harvested between 8 and 9 a.m. and immediately transported to the laboratory for examination. The following determinations were perfor-

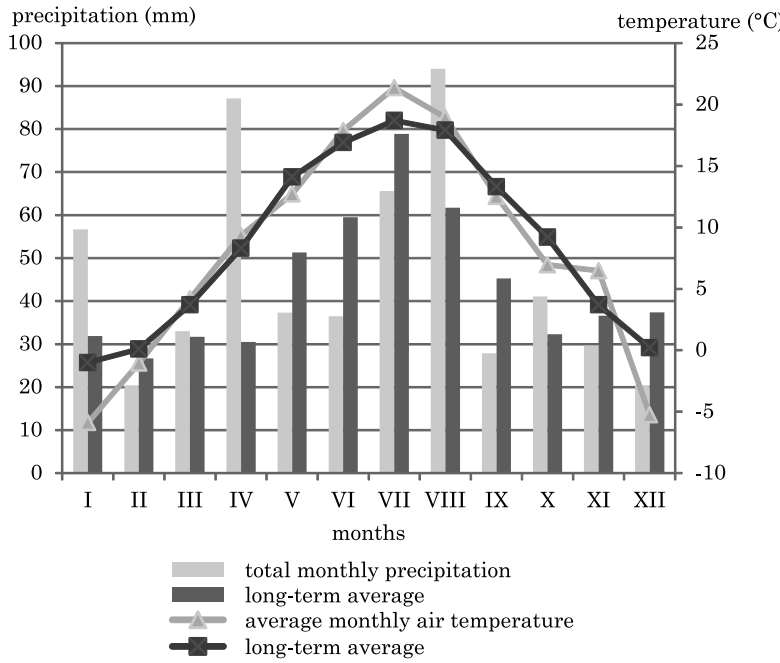

Fig 1. Weather conditions in 2010 against long-term averages

med: the activity of acid phosphatases (AP; EC 3.1.3.2), the phosphate 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.

BIOCHEMICAL ANALYSES

Extraction and determination of acid phosphatase

For extraction of acid phosphatase, 0.5 g of fresh plant material was ground in a cold mortar with 5 cm^3 of acetate buffer. After centrifugation (12 000 g for 10 min at 4°C), the supernatant was used for the determination of enzyme activity using 4-nitrophenyl phosphate (4-NPP) as an enzyme substrate. The enzymatic reaction consists in the conversion of 4-NPP into 4-nitro-phenol (4NP). The amount of 4-nitrophenol released was assayed colorimetrically after adding NaOH, while the absorbance of the samples was measured on a spectrophotometer at a wavelength 410 nm. The measure of enzyme activity is the amount of 4-NP released in 1 hour per 1 g of fresh weight (mmol (4-NP) g^{-1} fresh weight h^{-1}).

Extraction and determination of inorganic phosphorus

Inorganic phosphorus was extracted in 6% trichloroacetic acid (TCA) and the homogenates were centrifuged at 18 000 g for 15 min. Phosphate in the supernatant was determined colorimetrically after adding 5 mol dm^{-3} H_2SO_4, 2.5% ammonium molybdate and 0.25% 1,2,4-aminonaphtholsulfonic acid. After 15 min incubation at 37°C the colour intensity (blue) was measured at 660 nm.

Determination of photosynthetic pigments

Photosynthetic pigments were extracted using 80% acetone. The absorbance of the extracts was recorded at 470, 647, 663 nm and the concentrations of chlorophyll *a* (Chl *a*), chlorophyll b (Chl *b*), total chlorophyll (Chl *a* + *b*) and carotenoids were calculated using the LICHTENTHALER equations (1987). Additionally, the ratios Chl *a*/Chl *b* and (Chl *a* + *b*)/carotenoids were calculated.

Nutrient analysis

Plant material was dried and mineralised. Phosphorus and magnesium were determined using colorimetric assays with a vanadate-molybdate reagent for P and titan yellow for Mg. Potassium was measured with a flame photometer. Total nitrogen was determined using the Kjeldahl method.

Estimation of dry matter and sugar content in roots

These two parameters were determined in mature roots at the end of the plant growing season. The concentration of sugar was determined with the refractometric method. Dry matter was estimated after drying the roots at 105°C for two hours and then at 70°C to constant weight.

Statistical analysis

Statistical analysis was supported by the statistical package Statistica version 10. Analysis of variance was followed by the Duncan's test to identify homogenous groups within the means. Significant differences among treatments (control, laser primed, hydroprimed and a combination of hydropriming and laser radiation) were considered at the $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Phosphorus, after nitrogen, is the second most frequently limiting macronutrient for plant growth. Phosphorus is found in key molecules such as nucleic acids, phospholipids, dinucleotides and adenosine triphosphate. It makes up about 0.2% of a plant's dry weight. Phosphorus also plays a vital

role in controlling enzyme activity and hence in the regulation of metabolic pathways (SCHACHTMAN et al. 1998, WARAICH et al. 2011). Maintenance of stable cytoplasmic Pi concentrations is essential for many enzyme reactions. Our experiment has shown the highest concentration of phosphorus in all plants at the beginning of measurements, that is 75 days after sowing (DAS) – Table 1. In this period, plants emerged from seeds exposed to a combination of hydropriming and laser light contained statistically less Pi than the other plants. However, the phosphorus concentration in these plants remained relatively stable during the whole growing season and the differences in this parameter did not exceed 11% with respect to the first measurement. The mentioned change occurred at the third date of determinations, and it was the smallest in plants grown from seeds treated by laser light together with hydropriming. In the remaining plants (non-primed, laser primed, hydroprimed), changes were considerably bigger and a marked decrease was observed in the Pi concentration compared to the first set of determinations. The most severe decrease occurred in plants grown from seeds stimulated by laser irradiation, with the maximum decline by 50% relative to the first determinations observed at the third date (138 DAS) (Table 1). It is worth noting that the effect of different seed treatment on Pi concentrations at particular determination dates was relatively weak (except for laser irradiation in combination with hydropriming, as mentioned earlier). At the second and third dates, the highest levels of Pi occurred in HP+LP plants. The measured amounts of both soluble (Pi) and total phosphorus (Tables 1, 2) indicat-

Table 1

Effect of seed priming on acid phosphatase activity and phosphate concentration in leaves of sugar beet at different stages of growth and development

Treatment	Days after sowing				
	75	102	138	167	$LSD_{0.05}$
Phosphorus content (μg g^{-1} FW)					
Control	51.20 *a A*	36. 35 *b C*	29.43 *b D*	42.04 *a B*	6.90
Laser priming	49.71 *a A*	30.17 *c C*	24.73 *c C*	38.33*a B*	5.65
Hydropriming	48.20 *a A*	42.04 *a B*	26.46 *bc C*	41.55 *a B*	5.12
Hydropriming + laser priming	39.57 *b AB*	40.80 *a A*	35.12 *a B*	38.58 *a AB*	4.60
$LSD_{0.05}$	5.20	4.25	3.06	4.01	
Acid phosphatase activity (μmol(4-NP) g^{-1} FW h^{-1})					
Control	270.3 *a A*	160.8 *c B*	68.7 *c C*	82.8 *b C*	16.9
Laser priming	277.3 *a A*	171.1 *c B*	100.4 *a C*	97.7 *a C*	20.0
Hydropriming	232.8 *b A*	196.1 *b B*	92.2 *a C*	99.2 *a C*	16.3
Hydropriming + laser priming	218. 3 *b A*	235.5 *a A*	78.5 *b B*	72.3 *c B*	18.0
$LSD_{0.05}$	23.9	15.6	9.5	8.5	

Significant differences in values between the seed treatments at each date of determinations are followed by different small letters. Capital letters within a row indicate statistical differences in mean values during the plant growing season (at $P \leq 0.05$).

Table 2

Effect of seed priming on the concentration of some minerals in leaves and roots of sugar beet at different stages of growth and development

Treatment	Nutrients (g kg DW)							
	nitrogen		phosphorus		potassium		magnesium	
	leaves	roots	leaves	roots	leaves	roots	leaves	roots
	75 days after sowing							
Control	31.05 b	10.44 b	1.70	0.90	32.20 c	13.00 b	12.12 b	2.00
Laser primed	31.15 *b*	11.15 *a*	1.75	0.79	33.80 *ab*	13.35 *ab*	12.25 *b*	2.05
Hydroprimed	33.10 *a*	11.04 *a*	1.70	0.83	34.90 *a*	14.10 *a*	12.85 *a*	2.15
Hydroprimed + laser primed	30.15 *b*	10.90 *ab*	1.65	0.85	33.15 *bc*	13.55 *ab*	12.22 *b*	2.00
$LSD_{0.05}$	1.05	0.55	ns	ns	1.25	1.05	0.44	ns
	102 days after sowing							
Control	22.05 *c*	8.10	1.80	1.85	34.16 *b*	8.50 *bc*	13.40 *a*	2.00
Laser primed	23.15 *b*	8.00	1.90	1.80	35.65 *a*	8.45 *c*	13.35 *a*	2.15
Hydroprimed	24.10 *b*	8.37	1.90	1.80	35.45 *a*	9.25 *a*	12.85 *b*	2.74
Hydroprimed + laser primed	28.11 *a*	8.04	1.95	1.95	36.05 *a*	9.00 *ab*	12.22 *c*	2.50
$LSD_{0.05}$	1.10	ns	ns	ns	1.25	0.55	0.35	ns
	138 days after sowing							
Control	21.55*ab*	7.70	2.00	1.95	27.47 *b*	8.50 *b*	9.70 *c*	2.04
Laser primed	23.08 *a*	7.65	2.05	1.85	28.96 *a*	8.50 *b*	10.00 *b*	2.35
Hydroprimed	23.05 *a*	7.80	2.10	2.00	29.25 *a*	9.25 *a*	10.55 *a*	2.05
Hydroprimed + laser primed	19.50 *b*	7.40	2.28	2.00	29.85 *a*	9.00 *a*	10.15 *ab*	2.00
$LSD_{0.05}$	2.25	ns	ns	ns	1.28	0.35	0.42	ns
	167 days after sowing							
Control	17.83 *b*	6.70	1.90	1.85	25.85 *b*	8.55	8.56 *bc*	2.20
Laser primed	19.14 *ab*	7.00	2.08	1.86	27.96 *a*	8.00	8.36 *c*	2.18
Hydroprimed	20.05 *a*	7.05	1.95	2.00	29.03 *a*	8.05	9.15 *a*	2.30
Hydroprimed + laser primed	19.50 *ab*	7.05	1.90	2.00	29.50 *a*	8.15	8.85 *ab*	2.35
$LSD_{0.05}$	1.80	ns	ns	ns	1.58	ns	0.45	ns

Values in a column followed by different letters are significantly different at $P \leq 0.05$, ns – not significant

ed that all plants contained high and quite stable concentration of phosphate and neither the Pi uptake nor its translocation to the shoots was affected. Concluding, phosphorus metabolism in leaf cells and other important pro-

Table 3

Effect of seed priming on the concentration of photosynthetic pigments in leaves of sugar beet at different stages of growth and development

Treatment	Chlorophyll (mg g^{-1} FW)			Carotenoids (mg g^{-1}FW)	Chlorophyll *a*/*b* ratio	Chlorophyll/ Carotenoids ratio
	Chl *a*	Chl *b*	total			
75 days after sowing						
Control	1.003 *a*	0.309 *a*	1.312 a	0.322 *a*	3.25 *ab*	4.07 *b*
Laser priming	1.068 *a*	0.327 *a*	1.395 a	0.334 *a*	3.27 *a*	4.21 *ab*
Hydropriming	1.019 *a*	0.315 *a*	1.334 a	0.306 *a*	3.23 *ab*	4.36 *a*
Hydropriming + laser priming	1.005 *a*	0.325 *a*	1.330 a	0.311 *a*	3.09 *b*	4.28 *ab*
$LSD_{0.05}$	0.105	0.031	0.140	0.029	0.16	0.28
102 days after sowing						
Control	1.079 *b*	0.297 *b*	1.375	0.315 *b*	3.63	4.36
Laser priming	1.081 *b*	0.305 *ab*	1.386	0.302 *b*	3.64	4.58
Hydropriming	1.199 *a*	0.327 *ab*	1.526 a	0.351 *a*	3.67	4.36
Hydropriming + laser priming	1.219 *a*	0.335 *a*	1.554 a	0.350 *a*	3.65	4.43
$LSD_{0.05}$	0.110	0.300	0.145	0.031	0.15	0.24
138 days after sowing						
Control	1.155 *b*	0.335 *c*	1.490 *b*	0.365 *bc*	3.48	4.08 *b*
Laser primed	1.191 *b*	0.339 *bc*	1.530 *b*	0.352 *c*	3.53	4.38 *ab*
Hydroprimed	1.333 *a*	0.383 *a*	1.716 *a*	0.392 *ab*	3.50	4.39 *a*
Hydroprimed + laser primed	1.320 *a*	0.362 *ab*	1.685 *a*	0.402 *a*	3.65	4.20 *ab*
$LSD_{0.05}$	0.120	0.270	0.155	0.032	0.26	0.30
167 days after sowing						
Control	1.274 *b*	0.362 *b*	1.636 *b*	0.411 *a*	3.52 *a*	3.98
Laser primed	1.294 *ab*	0.400 *a*	1.694 *ab*	0.399 *a*	3.16 *c*	4.25
Hydroprimed	1.387 *a*	0.425 *a*	1.815 *a*	0.426 *a*	3.26 *bc*	4.26
Hydroprimed + laser primed	1.234 *b*	0.350 *b*	1.585 *b*	0.398 *a*	3.53 *a*	3.98
$LSD_{0.05}$	0.112	0.033	0.161	0.034	0.26	0.29

Values in a column followed by different letters are significantly different at $P \leq 0.05$.

cesses that require Pi (for example storage and transfer of energy, photosynthesis, the regulation of the activity of some enzymes, transport of carbohydrates) could proceed undistrubed. The key role in phosphorus metabolism and in controlling the amount of absorbed phosphorus and its circulation in

plants is played by acid phosphatases (orthophosphoric-monoester phosphohydrolases, EC 3.1.3.2) (Duff et al. 1994). These enzymes are involved in the metabolic processes of germination and maturation of plants. They are widespread in the plant kingdom and catalyse the hydrolysis of phosphorus compounds (Duff et al. 1994). Their amount and activity in the tissues depends on the content of phosphate in the cell. The hydrolysis of phosphate esters is an important process in energy metabolism, metabolic regulation and a wide variety of cellular signal transduction pathways of plant cells. Our experiments indicated that changes in the activity of acid phosphatases during the growing season were slightly similar to those observed in the amounts of phosphate in tissue (Table 1). The highest activity was observed at the first date of measurements and among different treatments, plants raised from non-primed seeds and laser primed seeds were characterized by a higher acid phosphatases activity than the other plants. The enzyme activity in plants grown from hydroprimed and both laser- and hydroprimed seeds was significantly lower than in the control and laser-primed plants. With the exception of the first period of measurements, all seed treatment methods caused an increase in the acid phosphatases activity in sugar beet leaves. At the end of the growing season (138 DAS), the activity of acid phosphatases lowered to approximately 1/3 of the activity at the beginning of the experiment (75 DAS), and the highest activity was observed in plants raised from hydroprimed seeds. Regarding the phosphorus content, its decline at the same time was smaller (maximum 23% in laser-treated plants). Considering the effect of different seed treatments on enzyme activity, both hydropriming and laser priming caused a significant increase in enzyme activity comparing to the non-primed plants (with the exception of plants at 75 DAS).

There are opinions that both laser irradiation and hydropriming may accelerate plant metabolis, improve the nutrient status and induce an increase in chlorophyll concentrations (Truchliński et al. 2002, Chen et al. 2005, Sacała et al. 2012, Prośba-Białczyk et al. 2013). Our experiments showed (Table 3) that concentrations of photosynthetic pigments and other associated indices (ratios of Chl *a*/Chl *b* and total chlorophyll/carotenoids), regardless of the seed treatments, remained on a similar level at the first date (75 DAS). However, during the later stages of growth, plants grown from hydroprimed seeds and both hydro- and laser-primed ones contained more photosynthetic pigments (particularly chlorophylls) than the other plants. The percent increment was over 10% in comparison to the control plants (untreated plants). A high concentration of chlorophyll may improve the photosynthetic rate and biomass production. It is also indicative of plants being well-supplied with nitrogen. During the growing season, plants maintained high concentration of photosynthetic pigments and chlorophyll was not destroyed. In most cases, changes in the Chl *a*/Chl *b* as well as in the chlorophyll/carotenoids ratios were insignificant. Generally, all the seed treatments either had a positive effect or had no effect on mineral composition (N, P, K, Mg) in leaves and roots during the growing season (Table 2).

The biggest changes in leaves were observed for nitrogen and potassium. In roots, the changes were smaller. Results obtained by other authors on various plants are inconclusive (Truchliński et al. 2002, Ćwintal et al. 2010). The data presented in Table 4 show that almost all the examined methods of

Table 4

Effect of seed priming on the accumulation of dry matter and sugar content in roots of sugar beet at the end of the plant growing period

	Yields of roots (t ha^{-1})	Sucrose (%)	Dry matter (%)
Control	67.2 *b*	16.9 *c*	24.7 *c*
Laser primed	72.7 *a*	17.4 *ab*	25.5 *a*
Hydroprimed	74.3 *a*	17.7 *a*	25.6 *a*
Hydroprimed+laser primed	69.5 *b*	17.1 *bc*	25.1 *b*
$LSD_{0.05}$	2.8	0,45	0.35

Values in a column followed by different letters are significantly different at $P \leq 0.05$.

the seed stimulation (laser irradiation, hydropriming and a combination of hydropriming and laser radiation) had a positive effect on the yielding parameters of the roots of sugar beet. The highest increase in the yield of roots, accumulation of dry matter and sugar content occurred in plants grown from hydroprimed seeds or treated by laser irradiation. The positive impact of seed stimulation on sugar beet productivity has been also implicated by others (Pietruszewski, Wójcik 2000, Wójcik et al. 2004, Rochalska et al. 2009).

CONCLUSIONS

1. Among the presented methods of seed treatment, hydropriming alone or in combination with laser irradiation caused the biggest changes in the examined parameters, particularly in the activity of acid phosphatase, photosynthetic pigment concentration and magnesium content in leaves.

2. Hydropriming promoted the accumulation of both chlorophyll *a* and chlorophyll *b* as well as total nitrogen in leaves of sugar beet.

3. All seed treatments caused an increase in the potassium content in leaves during the whole plant growing season.

4. The examined methods of seed treatment significantly improved the productivity of sugar beet (yield of roots, sucrose content and accumulation of dry matter in roots).

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