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THE EFFECT OF PRE-SOWING SEED STIMULATION ON THE GERMINATION AND PIGMENT CONTENT IN SUGAR BEET (*BETA VULGARIS* L.) SEEDLINGS LEAVES

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Key words: pigments, sugar beet, germination, laser radiation, magnetic field.

Abstract

Two methods were used in a laboratory experiment to assess the effect of stimulation of sugar beet seed clusters. Four duration times of exposure to magnetic field (MF) were used: P_1 (10 s), P_2 (30 s), P_3 (60 s) and P_4 (300 s) and two different doses of semiconductor laser radiation (LR) applied (five-and ten-fold irradiation by the basic dose of 0.25 J cm^{-2}, respectively). Subsequently, the sowing value of the seeds, the morphological traits of the seedlings and pigment content in their leaves were assessed. Stimulation of the seed germination energy and capacity was recorded. Seed vigor, their germination speed and germination time improved significantly under the influence of MF. The radicle elongated after both treatments. The pigment condensation in seedlings was stimulated most intensively by the lower of the two LR doses used. The obtained results allow the statement that magnetic field and laser radiation modify the germination process of sugar beet.

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WPŁYW PRZEDSIEWNEJ STYMULACJI NA KIEŁKOWANIE I ZAWARTOŚĆ BARWNIKÓW W LIŚCIENIACH BURAKA CUKROWEGO (*BETA VULGARIS* L.)

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Słowa kluczowe: barwniki, burak cukrowy, kiełkowanie, promieniowanie laserowe, pole magnetyczne.

Abstrakt

W doświadczeniu laboratoryjnym oceniano efekty stymulacji kłębków buraka cukrowego dwiema metodami. Zastosowano cztery ekspozycje pola magnetycznego (MF) P_1 (10 s), P_2 (30 s), P_3 (60 s) i P_4 (300 s) oraz dwie zróżnicowane dawki promieniowania lasera półprzewodnikowego (5-krotną i 10-krotną, dawka podstawowa naświetlenia wynosiła 0,25 J cm^{-2}). Oceniano wartość siewną nasion, cechy morfologiczne siewek oraz zawartość barwników w liściach siewek. Obserwowano wpływ badanych czynników na stymulację energii i zdolności kiełkowania. Wigor nasion, szybkość i czas kiełkowania istotnie się poprawiły pod wpływem działania pola magnetycznego. Korzeń zarodkowy wydłużył się po zastosowaniu obu sposobów traktowania nasion. Kondensację badanych barwników w największym stopniu stymulowało 5-krotne naświetlanie promieniami lasera.

Introduction

The high seed quality is decisive on the yield quantity and quality (ZAHOORET al. 2007). Therefore seed refining became a commonly applied, indispensable treatment, that aims at enhancement of the seedsvigour and reduction of the variability of their physical and chemical characteristics. At the present time, the chemical, as well as physical and physiological methods of seed refining are known (ANDREOLI and KHAN 2000, GRZESIK at al. 2012, JAKUBOWSKI 2015, JALALI and SALEHI 2013) The physical methods were developed by the end of the last century) (LIPSKI et al. 1996, ALADJADJIYAN 2007, DELIBALTOVA and IVANOVA 2006).

The most important among them are: the irradiation by the ionizing and microwave radiation (WÓJCIK et al. 2004) application of the constant and alternating magnetic field (PIETRUSZEWSKI 1992, PROKOP et al. 2002a, 2002b) and treating of the seed material with laser radiation (GŁADYSZEWSKA 2011, HERNANDEZ et al. 2008, SZAJSNER 2009 SZAJSNER et al. 2014).The application of these methods is legitimate on the merits of pro-ecological actions: although the magnetic field is no hazard to the environment, it may improve the seed vigour through the enhancement of the physiological processes in plant, and may as such be used as an auxiliary factor, supplementing other methods of seed refining (ROCHALSKA 2002a) or cultivation especially under stress conditions (PEJIC et al. 2011). Magnetic fields, especially those of low frequency, affect positively plant development as they stimulate transport of chemical substances in a plant. According to ROCHALSKA (2002a) this effect takes place through the alteration of the opening patterns of ion channels. The influence of magnetic field was investigated in many species of agricultural and horticultural plants, including vegetables and arboricultures. Pre-sowing treatment of the seed material with laser radiation exerts positive effect on the seed germination, the initial plant development and on the quantity and quality of yield in cereal plants (SZAJSNER 2009), as well as in vegetables (DROZD and SZAJSNER 2007), root crops (SACAŁA et al. 2012) and in legumes (PODLEŚNY 2002), with no concurrent detrimental effects on the natural environment.

First research in Poland on the seed treating with MF was conducted by KOPEĆ (1985). The materials in that study were the seeds of the sugar beet cultivar (cv.) PN Mono 3. The Early Plant Development (EPD) technology is a new method in seed refining of the sugar beet. Treating seeds with the EPD method makes it possible to obtain fast and regular emergence of plants and their accelerated development, both of which positively affect the plant yield. As demonstrated in many experiments with sugar beet seeds, the seed stimulation resulted in an increase of the mass of the store root and of the sugar content in it. It also enhanced the technological quality of the yield, as the water-soluble ash content was observed to be lower (WÓJCIK 2001).

The aim of the study was to investigate the effect of differentdoses of semiconductor laser radiation (LR) and magnetic fields (MF) on the initial growthand pigment content in sugar beet **(***Beta vulgaris* L.**)** seedlings leaves.

Material and Methods

Seed clusters of sugar beet cv. Alegra and cv. Sporta (Syngenta Sp. z o.o. Poland) were analysed in a laboratory experiment. The clusters were subject to two methods of stimulation: laser radiation (LR) at the doses D_5 and D_{10} (five- and ten-fold irradiation by the basic dose of 0.25 J cm^{-2}, respectively) and magnetic field (MF) of 30 mT induction. Four duration times of exposure to MF were used: P_1 (10 s), P_2 (30 s), P_3 (60 s) and P_4 (300 s). Laser stimulation was carried out using semiconductor laser (model CTL-1106MX, CTL – Centrum Techniki Laserowej – Laser Instruments Sp. z o.o. Poland), of 200 mW power and 670 nm wavelength. The irradiated fragment of the seed surface was determined by the CTL 1202 S scanner, coupled with the laser.

The irradiated as well as the non-treated (reference) seed clusters were sown in plastic trays. Cuvette lined with filter paper moistened with distilled water and placed in climate chamber MLR-351 H (SANYO Electric Co., Ltd. Japan). The experiment was established in four replicates of 50 seeds each.

The assessment of the seed parameters affecting their sowing quality: the germination energy (as % of normal seedlings – 4 days after sowing) and capacity (14 days after sowing) was carried out in laboratory conditions, according to the method proposed by ISTA (2008). The vigor index of the control and stimulatedseed clusters was calculated as the product of the mean sprout length (cm) multiplied by the mean germination capacity [%] according to PANASIEWICZ (2008). The speed germination index was calculated according to Maguire, (JAKUBOWSKI 2015) as the sum of the quotients of the number of normally germinated seeds to the number of days elapsed since the sowing date until the removal of the seeds from the tray.

m – number of the normally germinated seeds;

d – number of days elapsed since the sowing date until the removal of the seeds from the tray

$$\text{Maguire index} = \frac{m_1}{d_1} + \frac{m_2}{d_2} + \ldots + \frac{m_n}{d_n}.$$

The index of the average germination time of one seed according to Pieper (JAKUBOWSKI 2015) was calculated as the quotient of the sum of products of the number of normally germinated seeds on a particular day and the number of days elapsed since the sowing date until the removal of the seeds from the tray (*i.e.* the numerator in the formula), to the sum of these days (*i.e.* the denominator in the formula).

$$\text{Pieper index} = \frac{(m_1 d_1 + m_2 d_2 + \ldots + m_n d_n)}{(m_1 + m_2 + \ldots + m_n)}$$

The mean number of seeds germinated per day, according to Kotowski, (JAKUBOWSKI 2015) was calculated as the quotient of the sum of normally germinated seeds, to the sum of products of the number of normally germinated seeds on a particular dayand the number of days elapsed since the sowing date until the removal of the seeds from the tray.

$$\text{Kotowski index} = \frac{(m_1 + m_2 + ... + m_n)}{(m_1 d_1 + m_2 d_2 + ... + m_n d_n)}$$

Furthermore, the morphological traits of the seedlings such as the length of the radicle, the length of the epigeal portion of the seedling and that of cotyledon, were compared, and the alterations in carotenoids and chlorophyll pigment content in the seedling leaves were assessed.

Photosynthetic pigments extraction and determination.

Plant material (0.2 g of cotyledons) was homogenized in chilled 80% (v/v) acetone and then centrifuged at 5000 × g for 10 min. at 4°C. The obtained supernatant was collected and put to a flask a 25 cm^3 and filled by 80% acetone to a final volume 25 cm^3. The absorbance of obtained extracts was recorded at 470, 647, 663 nm (Cecil Aurius Series CE 2011 Visible Spectrophotometer (Cecil Instruments Limited, Milton, Cambridge, Great Britain)) and the concentrations of chlorophyll a, of chlorophyll b, total chlorophyll (chlorophyll *a* + chlorophyll b) and carotenoids were calculated using LICHTENTHALER (1987) and LICHTENTHALER and BUSCHMAN (2001) equations.

The study was carried out according to commonly recognized standards of laboratory experiments. The data obtained were analysed by two-way ANOVA (The statistical analysis of the results was performed with the STATISTICA 10.0 software (Stat Soft®, Inc., USA), with cultivars and the methods of stimulation applied to seed clusters as two experimental factors. The significance of the two sources of variation was tested with Snedecor *F* test, whereas the homogenous groups were determined using Duncan's multiple comparison test.

Results

The control plants of both varieties which were tested did not differ significantly from each other with regard to energy and germination capacity (Figure 1).

The LR and the MF applied in the experiment enhanced significantly the values of the germination energy and capacity, compared to the reference seeds. Although the longest exposure to MF, P4, resulted in a significant reduction of germination energy compared to reference (Figure 2*a*). For germination all variants caused a stimulating effect (Figure 2*b*). Using P_1, P_2 and P_3 exposure times caused an increase in that same parameter value in Alegra cv. On the other hand, in Sporta cv. such stimulation was observed as the effectof the MF exposure defined as P_2, P_3 and P_4 (Figure 3). Moreover, the P_1, P_2, P_3 and P_4 exposures resulted in a significant enhancement of the vigor in the examined sugar beet (both varieties) seeds (Figure 4).

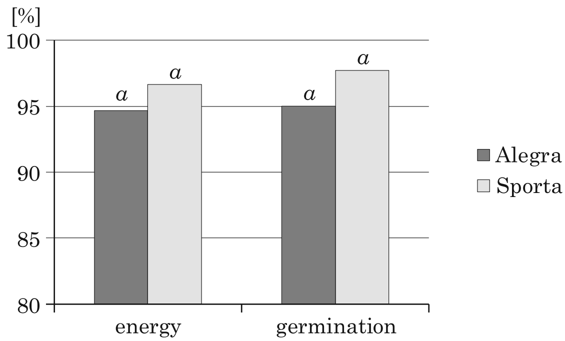

Fig. 1. Energy and germination capacity for control: $LSD_{\alpha = 0.05}$; energy = 3.75; germination = 4.15

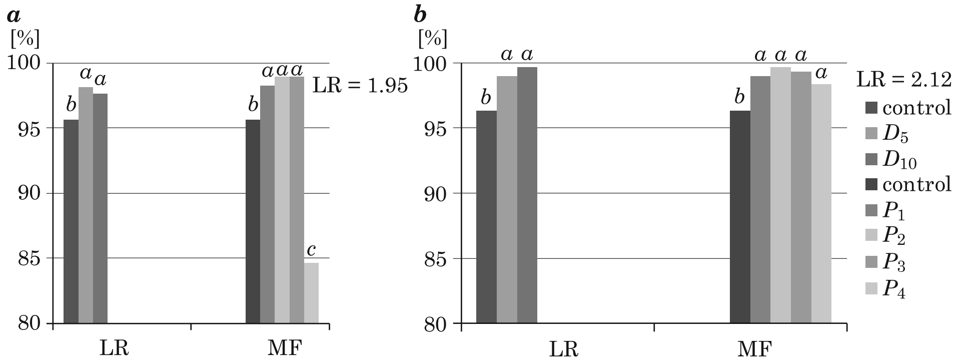

Fig. 2. The energy (a) and germination capacity (b) – average value.: $LSD_{\alpha = 0.05}$; MF = 1.71

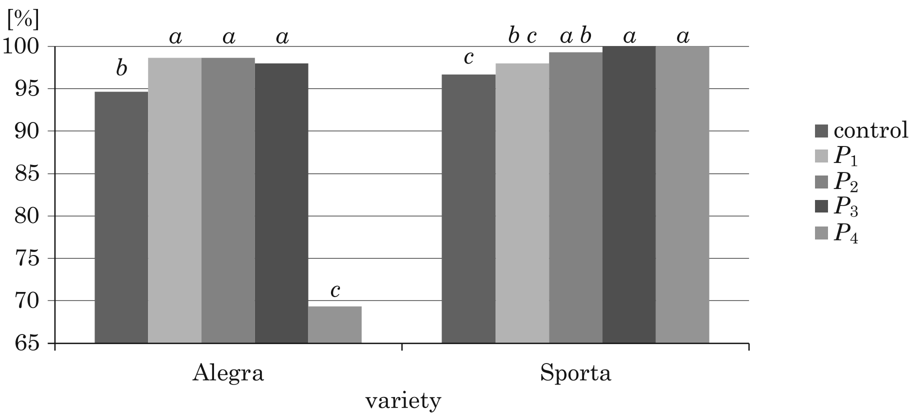

Fig. 3. The effect of stimulation (MF) on germination energy – interaction (variety x doses): $LSD_{\alpha = 0.05}$ = 2.41

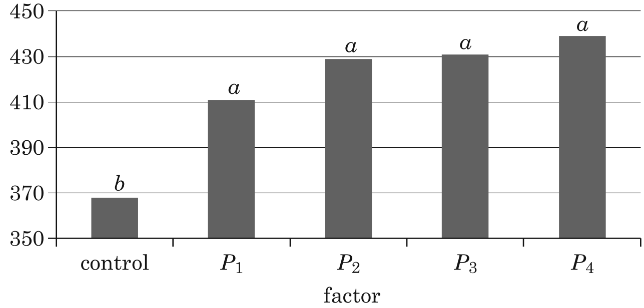

Fig. 4. The effect of stimulation (MF) on vigor – average value: $LSD_{\alpha = 0.05} = 37.4$

Germination indices were calculated as well for both varieties. Pieper index, giving the average germination time per one seed indicates that the dose of D_{10}, seeds need longer time to germinate but a dose D_5 significantly shortens the time. Based on the indices of Kotowski and Maguire it may be concluded that, the D_{10} dose decreased the average number of germinated seeds, and D_5 germination rate significantly (Figure 5).

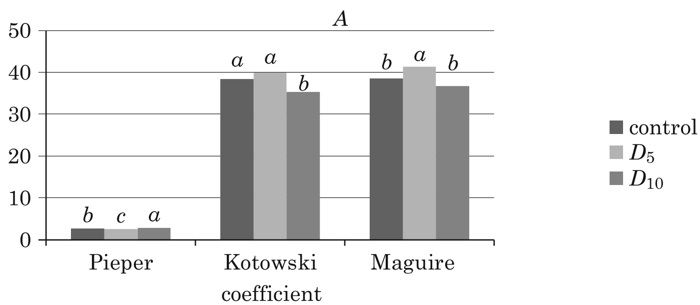

Fig. 5. The coefficient of germination – average value: $LSD_{\alpha = 0.05}$; Pieper = 0.129; Kotowski = 1.969; Maguire = 2.662

The interaction calculated for Pieper index allowed for conclusion that,use of magnetic field P_4 resulted in elongation whereas application P_3 shorter germination Alegry cv., Maguire index showed a significant increase in the average number of seeds germinated after application fields P_2 and P_3. In Sporta cv. no significant effects were observed of none of the applied pre-sowing seed stimulation techniques (Figure 6).

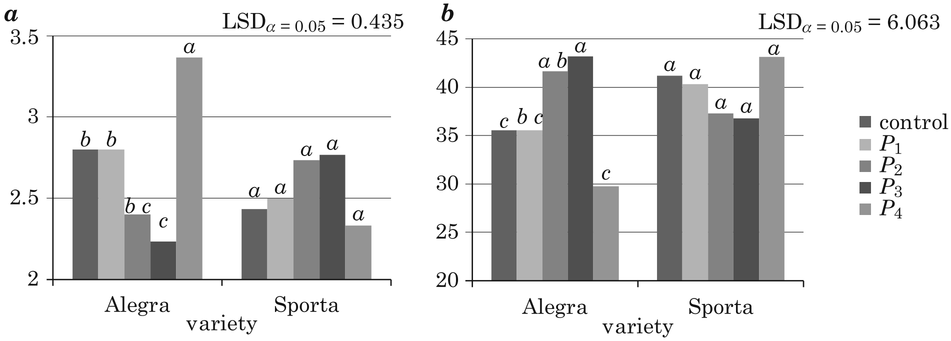

Fig. 6. The coefficient of germination to Pieper (*a*) and Kotowski (*b*) (MF) – interaction (variety x doses)

The seedlings of Alegra cv. produced shorter radicles and cotyledons. Significant stimulation (for two varieties) was observed in radicle length trait after each one of the treatments applied: the effect oscillated between the length enhancement by 20.2 mm after the P_1 duration of MF exposure and the enhancement by > 20.4 mm after the D_{10} dose of LR (Figure 7*a*).

The seeds of Sporta cv. showed higher sensitivity, the LR applied, with respect to the length of radicleby 34.8 mm. Alegra cv. did not react to the laser light (Figure 8).

When compared to the cotyledons of the reference seedlings, the seedlings grown from the seeds exposed to D_5 dose of LR and to the MF through the P_1, P_3 and P_4 durations, had shown significant stimulation of the cotyledon length (Figure 7*b*).The length of the seedlings (both varieties) was extended only under the influence of the MF – P_2, P_3 and P_4 (Figure 7*c*).

Statistical analysis indicated significant variation of the content of particular pigments in the leaves of the examined seedlings, after application of the pre-sowing seed stimulation techniques. The highest content of carotenoid pigments, compared to the reference plants, was found in the seedlings grown from the D_5 – and P_1 treated seeds (LR and MF respectively). An increase in the carotenoid content was also observed after P_3 duration of MF (Figure 9).

In Alegra cv., the stimulating effect of the five-fold LR (D_5). In Sporta cv., an increase in carotenoid content was observed after five- and ten- folddose of LR (Figure 10).

Chlorophyll a content increased significantly too as the effect of D_5 dose of LR and MF applied over P_3 and P_4 exposure times. Analysis of the data obtained also revealed an interaction of cultivars and treatments. The content of chlorophyll a increased under the influence of all the factors used with the exception of MF P_4 (Figure 9).

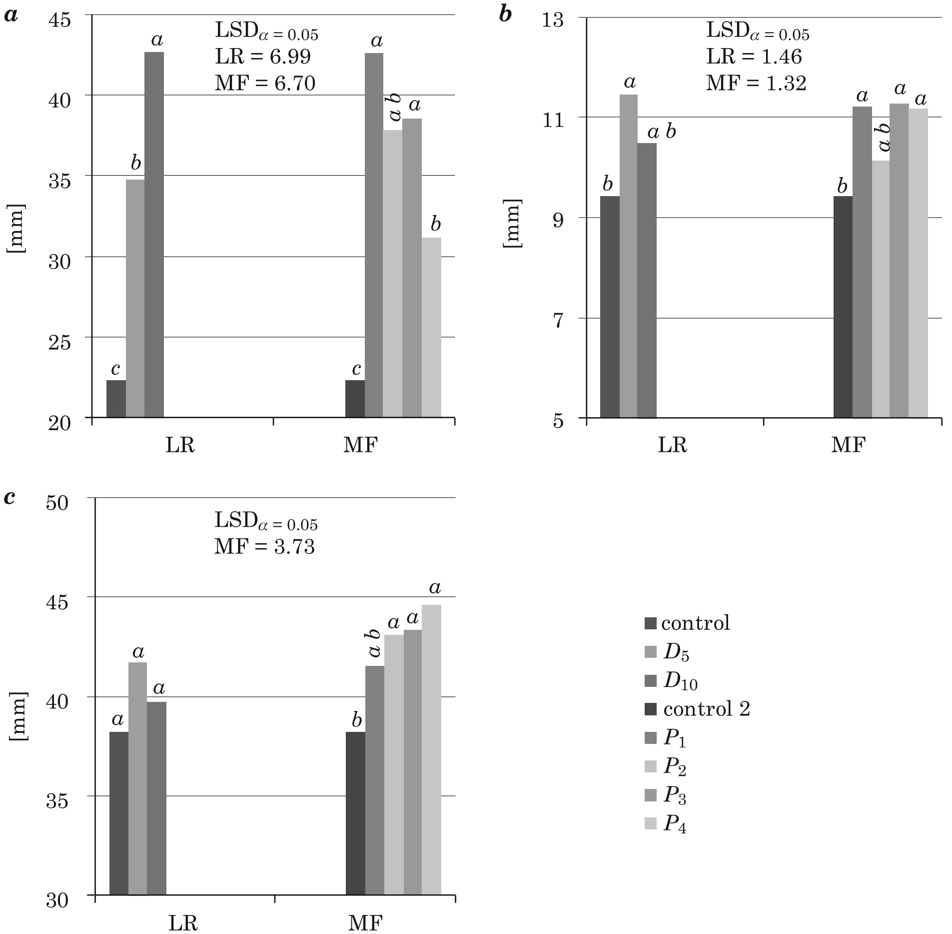

Fig. 7. Influence of seeds treatment for the root (*a*), cotyledon length (*b*) and seedling length (*c*) – average value

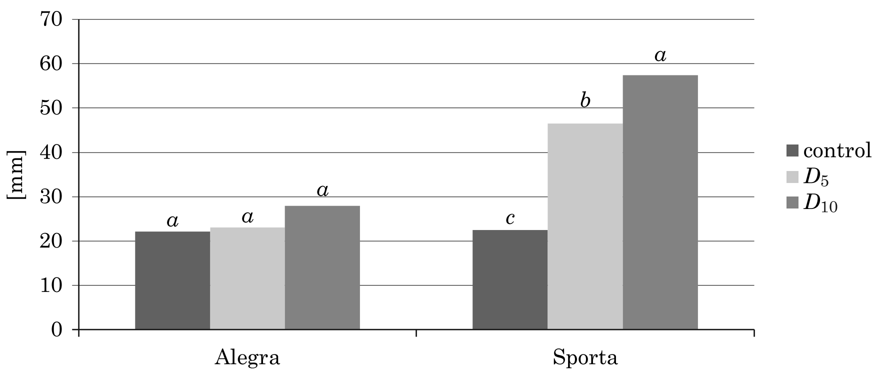

Fig. 8. The influence of laser radiation on root length – interaction (variety x doses): LSD$_{\alpha = 0.05}$; LR = 9.88

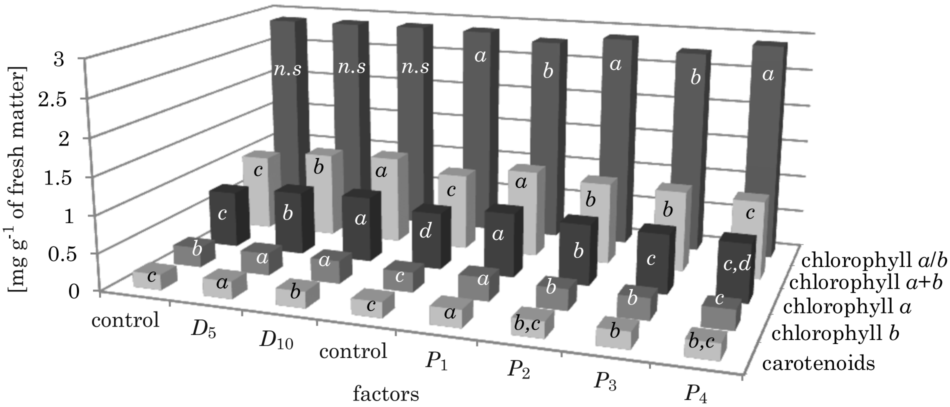

Fig. 9. The influence of pre-sowing stimulation on pigments content – average value: LSDα = 0.05 = LR; chl$a$ b^{-1} = n.s., MF = 0.0844; chl$a$ + b = 0.0250, MF = 0.0295; chl$a$ = 0.0179, MF = 0.0170; chl$b$; = 0.0126, MF = 0.0121; carotenoids = 0.0039, MF = 0.0121; a, b, c – homogeneous groups

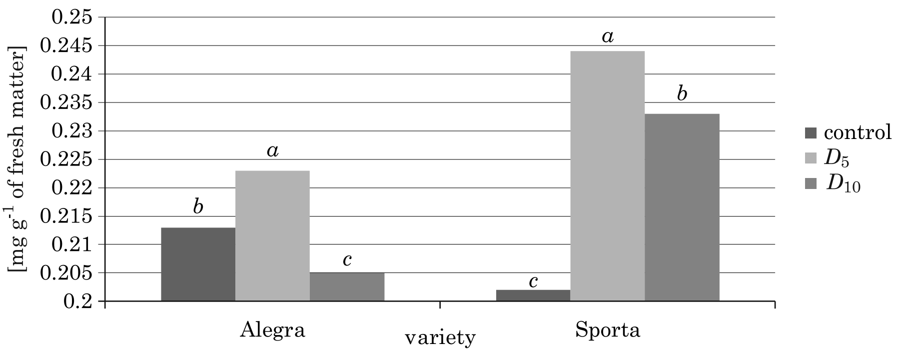

Fig. 10. The influence of pre-sowing stimulation on carotenoids pigments content – interaction (variety x doses): LSD$_{\alpha = 0.05}$ = 0.0056

Based on the interaction observed in a Alegra cv. increase in carotenoids under the influence of dose D_5, in Sparta cv. highest condensation observed under the influence of dose D_5, less affected dose D_{10} (Figure 10). The content in seedlings of the chlorophyllb increasedafter the seeds were treated with the D_5 and D_{10} dose of LR and MR – P_1, P_2 , P_3 (Figure 9). In seedlings grown from the seeds treated with both doses of LR and exposed to MF over all of the duration times except P_4, significant enhancement of chlorophyll a + b content was observed. The highest stimulation of the chlorophyll a + b content was obtained after D_{10} dose of LR and after P_1 of MR (Figure 9).

The interaction between cultivars and treatments demonstrated statistically significant stimulation of chlorophyll a content in Alegra cv., resultant from each one of the experimental factors applied, at all rates and over all of the exposure times.

In Sporta cv., the longer of the four MF exposure times: P_3 and P_4, significantly enhanced the concentration of chlorophyll a in the leaves of the seedlings.

The content of chlorophyll $a + b$ was stimulated in Alegra cv. compared to the reference plants, as the effect of all the applied treatments except P_4 exposure to MF. In Sporta cv. after treating the seeds with MF – P_3 and P_4.

The quotient of chlorophyll a to chlorophyll b oscillated between 2.64 and 2.96 depending on the applied treatment and the examined cultivar (Figure 11).

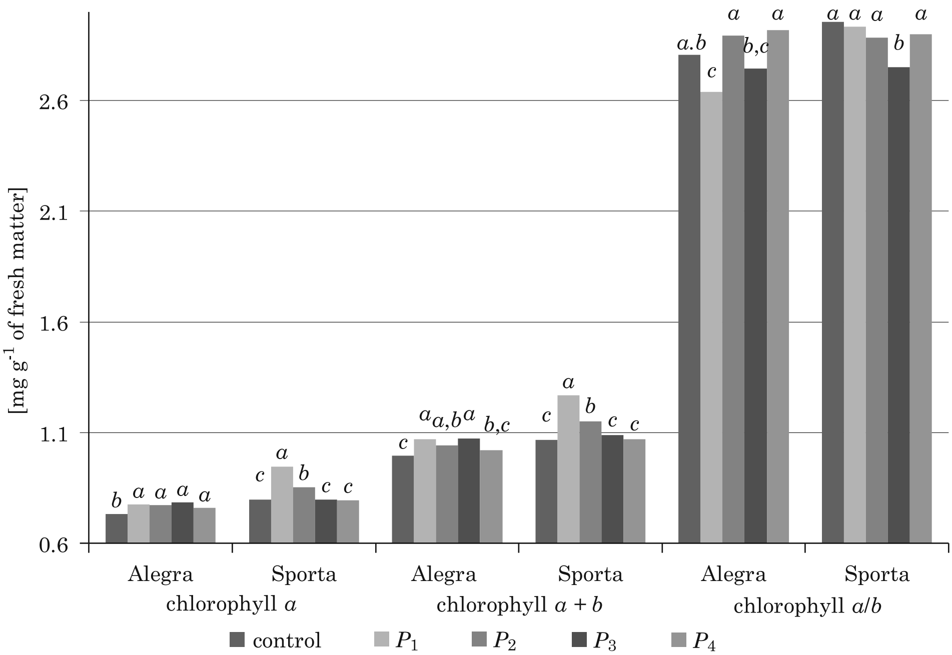

Fig. 11. The influence of pre-sowing stimulation on chlorophyll a, chlorophyll $a + b$ and chlorophyll a/b content – interaction (variety x doses): LSDα = 0.05; chl$a$ = 0.025; chl$a + b$ = 0.042; chla/b = 0.119

Discussion

Refining seed and other sowing materials by with LR treatment results in an enhancement of the treated material and an increase in quantity and quality of the yield obtained (PROŚBA-BIAŁCZYK et al. 2012)The experiments with sugar beet showed higher sugar content, and higher technological value: lower content of water-soluble ash and of melassigenic substances, in the roots of plants grown from the stimulated seeds (ROCHALSKA 2007).

Constant and alternating magnetic fields stimulate the process of seed germination, and plant development, as well as improve the yield and its quality (NAWROZ and HERO 2010). MARTINEZET al. (2001) have demonstrated that low intensity MF positively affects seed germination and early plant development. In contrary, high intensity MF may adversely affect plants, reducing the germination capacity of seeds and inhibiting plant growth.

The laser radiation and magnetic field used in the present experiment have, in general, increased germination energy and capacity significantly. Only the longest duration of the MF (P_4) was proved to be a reducing factor, as it had negatively affected seed germination energy. This last observation corresponds to and confirms the results of MARTINEZET al. (2001). Furthermore, the values of Pieper index also show extending of the germination time, after P_4 exposure to MF, in the seeds of one of the examined cultivars. A contrasting effect – the shortening of the seed germination time – was seen when the seeds were exposed to MF through a shorter period of time (P_3).

Both of the applied refining factors used in the experiment stimulated the length of the radicle, compared to the reference plants, by as much as 35 mm in the case of D_{10} LR dose. The cotyledons elongated after using D_5 LR and P_1, P_3 and P_4 MF exposure times. In an earlier research PROŚBA-BIAŁCZYK et al. (2013) had observed stimulation of the morphological traits in seedlings grown from the LR-stimulated seeds and originated in energỳ hill technology. Moreover, the higher content of carotenoid and chlorophyll pigments was obtained in the seedlings from stimulated seeds, and laser irradiation positively altered chlorophyll a to chlorophyll b ratio. While studying the effects of laser irradiation in the grain of maize, PODLEŚNY and STOCHMAL (2005) had observed the seed mass during imbibition stage being higher in the LR-exposed seeds compared to reference ones. The result of this phenomenon was germination that was earlier and more uniform in time, and faster growth and development of the seedlings. When compared to the seedlings grown from the non-treated seeds, those grown from the irradiated grain were characterized by longer sprout and roots. Data available in literature indicate that also magnetic field impels the germination process and is doing so by amplifying the enzymatic activity in the stimulated seeds. Seedsexposed to MF imbibe faster and start germinating more rapidly, which leads to the earlier and more regular plant emergence (PODLEŚNY et al. 2004). In the seedlings grown from the stimulated seeds longer stem and roots are observed, as well as higher plant vigour compared to the reference plants (PODLEŚNY 2004). In the experiments with a coupled application of LR and MF in the seeds of buckwheat, CIUPAK et al. (2007) reported the 7% increase in the number of seeds germinated at the initial germination stages.

The results obtained by GARCIA et al. (2013) concerning the application of MF in soybean seed refining evidenced the increase in the percentage of the germinated seeds and in the root length in the seedlings grown from the stimulated seeds. Seeds stimulated with the MF of 16 Hz and 50 Hz frequency and 5 mT induction through the exposure time of 2 h, while germinating under stress conditions in the temperature 5°C, had shown their germination capacity higher by ca. 20% and a considerable, by 1 day, shortening of the average germination time (ROCHALSKA 2002a). Exposing seeds of wheat to MF also increased their field emergence capacity, accelerated their development and enhanced the yield. This effect was particularly apparent in the case of older grain of the reduced qualitative parameters (ROCHALSKA 2002b). In the experiment on the wheat grain stimulation with MF, KORNARZYŃSKI and PIETRUSZEWSKI (2005) had found the greatest germination speed and the average relative germination speed in the seeds exposed to 15 mT induction magnetic field. Also CARBONELL et al. (2000) had found, in their research concerning rice and barley, that the germination of rice was faster and the yield of barley – higher – after the grain stimulation using MF.

PIETRUSZEWSKI (1993) had used alternating MF of 50 Hz frequency to stimulate spring wheat grain and determined an optimal dose at 30 mT induction with 4 sec. and 8 sec. exposure times. He had obtained yield increase by 24% and 20% for 4 sec. and 8 sec. exposures respectively in Henika cv., whereas the respective values in Jara cv. were 36% and 25%. Moreover, he had observed an increase in the ear and grain number, as well as in grain mass, compared to the reference (PIETRUSZEWSKI 1993). In maizere search (ALADJADJIYAN 2010) a more intensive stem growth or a yield increase compared to the reference plants were observed after 150 mT or 60–200 mT MF stimulation, respectively.

Biologically active light becomes absorbed by respective pigments. Carotenoids are photolabile, i.e. their content in a plant is dependent on the presence of light. They absorb light within the part of spectrum that is not absorbed by chlorophylls and subsequently convey the absorbed energy to chlorophyll molecules. They therefore have an auxiliary function in photosynthesis and play a protective role in the processes of photooxidation.

The five-fold laser irradiation of the seeds, used in the present experiment, has resulted in an increase of the content of all the examined pigments: carotenoids, chlorophyll a, chlorophyll b, as well as that of chlorophyll $a + b$, like in the study of seedlings of maize (HERNANDEZ at al. 2008). The magnetic field in the P_3 and P_4 exposure durations also affected the content of all the examined pigments, except for thechlorophyll b. While investigating the use of MF in sugar beet independently or along with the seed cluster abrasion and conditioning ROCHALSKA (2005) had found an increased chlorophyll content in plant leaves. In another study, using weak (0.5 mT) constant MF resulted in the increased leaf dimensions and enhanced the protein and chlorophyll content in the seedlings of onion (NOWITSKY et al. (2001).

Experimenting with the chick pea, NAWROZ and HERO (2010) had shown a positive influence of short-lasting seed exposures to MF on a number of morphological traits of seedlings and on the content of photosynthetically active pigments. The stimulated traits included stem and root length, the fresh and dry plant mass, and the content of chlorophyll *a* and *b* as well as that of carotenoids. These results comply well with the ones obtained by DHAWI and AL-KHAYRI (2001) in the study on the date palm (*Phoenix dactylifera* L.). These authors found that the content of chlorophyll and carotenoid pigments in plants had increased considerably as the effect of exposure to the constant MF, while it decreased after using the alternating MF. The content of photosynthetically active pigments was increasing as the effect of low MF doses, whereas the high MF doses triggered the opposite, negative effect. Furthermore, a greater variation in the condensation of chlorophyll *a* and of carotenoidswas observed, than of chlorophyll *b*.

Conclusions

1. Each of the two examined methods of pre-sowing seed stimulation significantly increased the values of germination energy and germination capacity of the seed clusters.
2. P_1, P_2, P_3 and P_4 exposures to magnetic field significantly improved seed vigour, whereas the germination speed (Maguire index) and germination time (Pieper index) were stimulated only by the D_5 dose.
3. The radicle length was stimulated significantly by each of the seed treating methods examined.
4. The highest concentration of the examined plant pigments was observed after five-fold laser irradiation (D_5). Magnetic field at P_3 exposure duration enhanced the content of carotenoids and chlorophylla.
5. The fife-fold laser irradiation (D_5) and P_3 exposure time to magnetic field proved to be the treatments most advantageous to the examined seed clusters.
6. The use of magnetic field and laser radiation may be the method used successfully in order to offset the rising and accelerate the growth of seedlings.

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