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INFLUENCE OF LASER STIMULATION SEEDS ON GERMINATION AND INITIAL GROWTH OF SEEDINGS *SILENE VULGARIS*

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Key words: *Silene vulgaris*, ecotype, morphological properties, laser light, seed germination, germination energy, seed germinability.

Abstract

The aim of research was to determine the effect of pre-sowing stimulation of seeds from selected ecotypes of *Silene vulgaris* with semiconductor laser beams on increased phytoremediating ability of plants. Three ecotypes of *Silene vulgaris,* originating from the area of natural (Gajków ecotype) and elevated content of heavy metals (Wiry and Szopienice ecotypes) were subjected to investigation. The following doses of laser light were applied: D_1 – single, D_3 – threefold, D_5 – fivefold, D_7 – sevenfold – and D_{10} – tenfold irradiation with basic dose amounting $2.5 \cdot 10^{-1}$J cm^{-2} and control variant C – seeds not subjected to irradiation. The ecotypes subjected to examination did significantly differ in their properties. It was possible to observe diverse response of the ecotypes to applied pre-sowing laser radiation. The improvement in the sowing value was obtained after application of doses D_1, D_3 and D_5, while stimulation of biometric traits of seedlings resulted from the introduction of doses D_3, D_7 and D_{10}. The two of them (Wiry and Gajków ecotypes) showed increased sowing value, as well as elongation of embryonic root and above – ground parts of seedlings. The ecotype originating from Szopienice proved to be insensitive to pre-sowing application of laser radiation

WPŁYW STYMULACJI LASEROWEJ NASION NA KIEŁKOWANIE I POCZĄTKOWY WZROST SIEWEK *SILENE VULGARIS*

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Słowa kluczowe: *Silene vulgaris*, ekotyp, cechy morfologiczne, światło lasera, kiełkowanie nasion, energia kiełkowania, zdolność kiełkowania.

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Abstract

Celem badań było określenie wpływu przedsiewnej stymulacji nasion *Silene vulgaris* promieniami lasera półprzewodnikowego na wartość siewną oraz cechy morfologiczne siewek. Stymulacja prowadziła do zwiększenia zdolności fitoremediacyjnych wybranych ekotypów. Badano trzy ekotypy *Silene vulgaris* pochodzące z obszarów o naturalnej (ekotyp Gajków) i podwyższonej zawartości metali ciężkich (ekotypy Szopienice i Wiry). Zastosowano następujące dawki światła laserowego: D_1 (jedno-), D_3 (trzy-), D_5 (pięcio-), D_7 (siedmio-) i D_{10} – dziesięciokrotne naświetlanie dawką podstawową wynoszącą $2.5 \cdot 10^{-1}$J cm^{-2}, czas naświetlania 4.1 min. oraz wariant kontrolny (*C*) – nasiona niepoddane naświetlaniu. Ekotypy różniły się istotnie pod względem badanych cech. Obserwowano zróżnicowaną reakcję na zastosowane przedsiewnie promieniowanie laserowe. Poprawę wartości siewnej otrzymano po zastosowaniu dawek D_1, D_3 i D_5, natomiast stymulację cech biometrycznych siewek pod wpływem D_3, D_7 i D_{10}. Dwa z badanych ekotypów (Wiry i Gajków) reagowały podwyższeniem wartości siewnej, jak również wydłużeniem korzeni zarodkowych i nadziemnych części siewek. Ekotyp pochodzący z Szopienic okazał się niewrażliwy na przedsiewne zastosowanie promieniowania laserowego.

Introduction

Plants growing on soils containing natural high contents of heavy metals have been the object of scientists interest for a long time. However, this interest has been focused on hyperaccumulators, i.e. species absorbing and storing heavy metals in considerable amounts in relation to their total weight (PROCTOR 2003, CHANEY et al. 2005, KAZAKOU et al. 2010). On the area of Poland there do not occur typical hyperaccumulators, yet some of native species characterize high tolerance to harmful effect of heavy metals. These species are called metalophytes. To this „group" of Polish metalophytes is classified *Silene vulgaris* (*Caryophyllaceae*) (WIERZBICKA and PANUFNIK 1998, KOSZELNIK-LESZEK 2012), growing on the areas secondarily enriched in heavy metals, as well as on soils featuring natural high content of heavy metals, such as Zn – Pb ore areas, ore or serpentine areas (WIERZBICKA and PANUFNIK 1998, KOSZELNIK-LESZEK 2012, ŻOŁNIERZ 2007, NADGÓRSKA-SOCHA et al. 2011). Unique adaptation abilities of this species (BRATTELER et al.2002) resulted in the development of separate ecotypes, capable of growing in extremely unfavorable conditions of their habitat. Apart from *S. vulgaris* ecotypes, resistant to lead (WIERZBICKA and PANUFNIK 1998, NADGÓRSKA-SOCHA et al. 2011, KANDZIORA et al. 2007), there are also known the ones which tolerate excessive amount of cadmium, zinc, copper (NADGÓRSKA-SOCHA et al. 2011, HARMENS et al. 1993, VERKLEJI and PRAST 1989) or arsenic and cobalt (PALIOURIS and HUTCHINSON 1990). Tolerance to Ni has been described as the example of non – specific co – tolerance of *S. vulgaris* to different metals (PALIOURIS and HUTCHINSON 1990, GABBRIELLI et al. 1990, WESTERBERGH 1994). Metalophytes, including *S. vulgaris*, are natural phytoremediators, used in the methods of soil remediation. This way of biological reclamation makes use of the proper-

ties of some plant species regarding absorption and accumulation of pollutants in their tissues, in the amounts exceeding those commonly found in tissues of other plants. The success of phytoremediation depends, first of all, on the choice of appropriate plant species. Therefore, they can prove to be especially valuable e.g. populations of *S. vulgaris,* whose natural resistance enables their growth in difficult habitat conditions, connected with excessive quantities of metals. Majority of naturally occurring phytoremediators are plants with poor weight gain, which are not always suitable to be widely used in a particular phytoremediatory method and, therefore, there was undertake research in order to determine the effect of pre-sowing stimulation of seeds of *Silene vulgaris* ecotypes with semiconductor laser beams on increased phytoremediatory abilities of the seedlings. Usefulness of plant stimulation with laser can be confirmed by apparent increase in plant biomass, acceleration of plant growth, as well as its influence on physiological processes (SACAŁA et al 2012, DANAILA-GUIDEA et al. 2011, PROŚBA-BIAŁCZYK et al. 2012), and increased content of biogenic elements in plant biomass (ŚLIWKA and JAKUBIAK 2009, ASHRAFIJOU et al. 2010, ŚLIWKA and JAKUBIAK 2010). Moreover, there were observed differences in accumulation of Cu, Cd, Ni in willow leaves, as well as lack of negative effect of these chemical elements on plants as their content increases (GRYGIERZEC and GOWIN 2010, JAKUBIAK and ŚLIWKA 2010).

Materials and Methods

General characteristics of selected ecotypes of *Silene vulgaris*

Gajków ecotype comes from natural habitat, from the village Gajków situated on south – east of Wrocław (Poland – Lower Silesia), (KOSZELNIK--LESZEK and WALL 2009).

Szopienice ecotype grows on the area situated 250 meters from pollutants emitter, i.e. Non – ferrous Metals Smelter „Szopienice" in Katowice (Poland – Upper Silesia) (NADGÓRSKA-SOCHA and CIEPAŁ 2009).

Wiry ecotype covers a small heap connected with exploitation of serpentine deposits in Lower Silesia near the village Wiry, located not far from western foothills of the Ślęża Mountain (KOSZELNIK-LESZEK 2007).

Analysis of seeds of selected *Silene vulgaris* ecotypes. The sizes of 25 randomly selected seeds from each of three *S. vulgaris* ecotypes were measured. The measurement [mm] was done in the widest and the narrowest site of each seed, using the microscope Axioskopu 2 plus, at magnitude 10x and program AxioVision 2.0.

Laboratory experiment with the use of laser radiation. The purpose of laboratory research was determination of the effect of treatment seeds with semiconductor laser beams on sowing value, as well as morphological character of seedlings originating from the examined ecotypes. Before establishing the experiment, seeds were exposed to laser light – semiconductor laser model CTL – 1106 MX. Applying the scanner (model CTL 1202 S), which cooperated with laser, there was determined radiation – exposed surface. There were applied the following doses of laser light: D_1 – single, D_3 threefold, D_5 – fivefold, D_7 – sevenfold and D_{10} – tenfold irradiation with basic dose amounting $2.5 \cdot 10^{-1}$J cm^{-2} , exposure time 4.1 min. and control variant C – seeds not subjected to irradiation. Seeds of control and irradiated with laser light were sown in the first day after exposure to plastic plates. The experiment was established according to the method of independent series. Sowing material of *S. vulgaris* was placed in a germination apparatus in controlled temperature and humidity conditions. There were estimated energy and germination capacity – according to International Rules for Seed Testing ISTA 2007. The measurements of morphological properties of seedlings grown from control and irradiated seeds included: the length of embryonic root, above – ground parts of seedlings and cotyledons.

The results obtained due to the measurements underwent statistical analysis according to the methodology appropriate for two – factorial laboratory experiment. The subject of the assessment were: significance of ecotypes diversity, doses of laser light, as well as interaction between these factors. Test F was used in order to determine significance of differences between the variants applied, while Duncan test was applied to form homogeneous groups.

Results and Discussion

The results of measurements of three selected seed S. *vulgaris* ecotypes are shown in Figure 1. The seeds have a natural population size which is a typical length intervals of 1.25 – 1.5 mm and a width of 2–1.25 mm and width 2–1.25 mm (MATUSZKIEWICZ 2008). The length of seeds from population coming from the areas polluted with heavy metals can be found within the lower limit of typical interval length, while seeds width are placed below lower limit model width of *S. vulgaris* seeds. Seeds of *Silene vulgaris* (Figure 2) natural population were larger than those coming from the areas of elevated content values of heavy metals and, therefore, they were probably richer in nutrients. *S. vulgaris* populations, growing in habitats contaminated with metals, developed seeds of lower, probably poorer in nutrients.

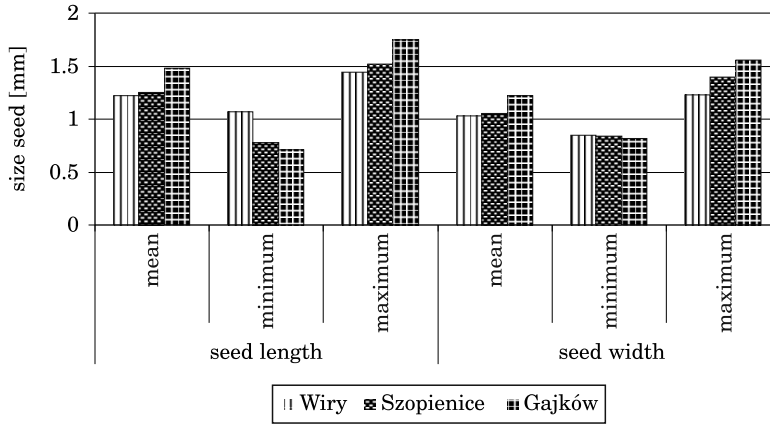

Fig. 1. Size seeds of selected ecotypes of *Silene vulgaris* [mm]

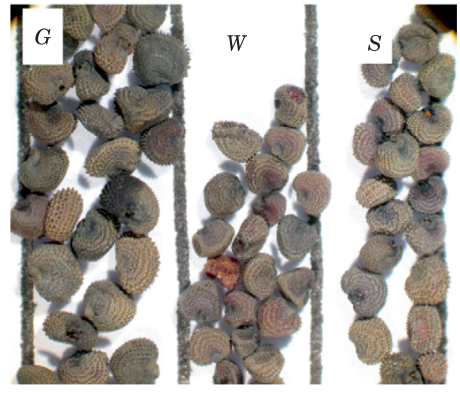

Fig. 2. Seeds of selected ecotypes of *Silene vulgaris: G* – Gajków, *W* – Wiry, *S* – Szopienice (microscope NIKON SMZ800)

Analysis of variance, applied to germination capacity, showed significant diversity of the examined ecotypes (Gajków, Wiry, Szopienice), as far as laser radiation doses were concerned. Sowing material of three investigated ecotypes did significantly differ in germination capacity. The highest value of this property belonged to the ecotype from Wiry (76.78%), lower value characterized the ecotype from Szopienice (49.61%), while the lowest one featured Gajków ecotype (41.56%) – Figure 3. Stimulating effect of pre-sowing seeds irradiation with laser beams was observed after application of dose D_5 (65.89%) as compared to control amounting 59.67%. Doses D_{10} and D_7 proved to be too high and resulted in reduction of the value of that property to 46.89 and

44.33% respectively (Figure 3). Interaction ecotype x dose allowed to state that the effect of germination capacity reduction in Gajków ecotype after introduction of doses D_1 (40.00%), D_{10} (30.00%) and D_7 (22.67%) in relation to control (56.67%). The response of Wiry ecotype to application of pre-sowing irradiation with doses D_5, D_3 and D_1 was stimulation – increase in the value of the mentioned property by 23.34; 18 and 14.67% respectively in comparison to germination capacity of control seeds, which ranged 71.33%. The ecotype from Szopienice did not show any response to irradiation with laser beams (Figure 3). Considering the length of embryonic root, conducted analysis of variance demonstrated significant diversity of the examined ecotypes, as well as interaction ecotype x dose. Significantly longer embryonic root characterized ecotypes: Gajków (42.60 mm) and the one originating from Wiry dump (41.82 mm), as compared to the ecotype from Szopienice (29.34 mm) – Figure 4.

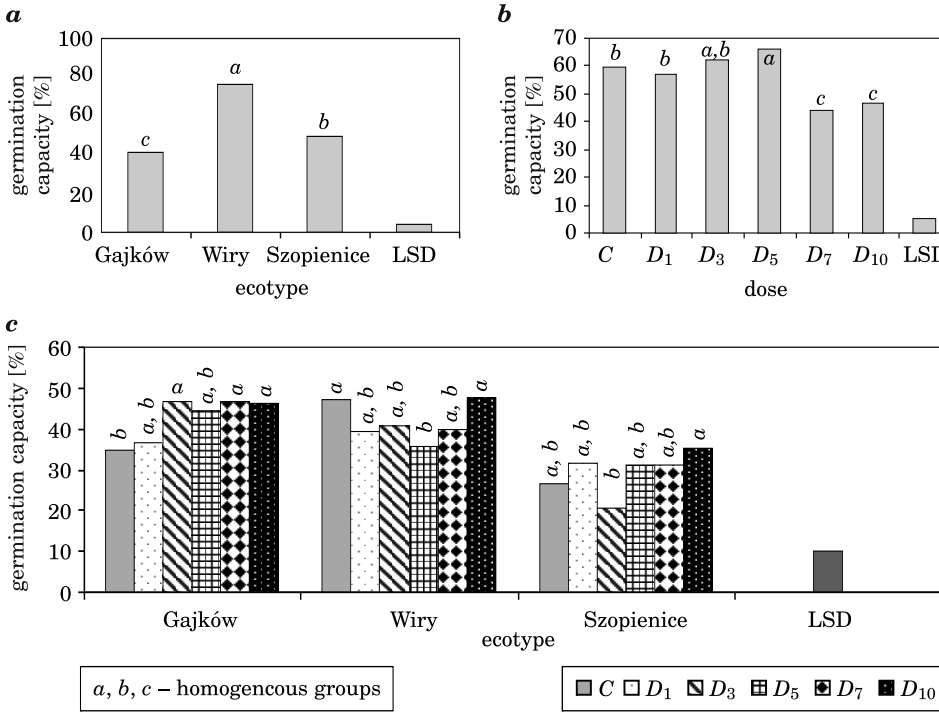

Fig. 3. Effect of stimulation of selected ecotypes of *Silene vulgaris* seeds on the germination capacity: *a* – ecotype; *b* – dose; *c* – interaction ecotype x dose; $LSD_{\alpha=0.05}$

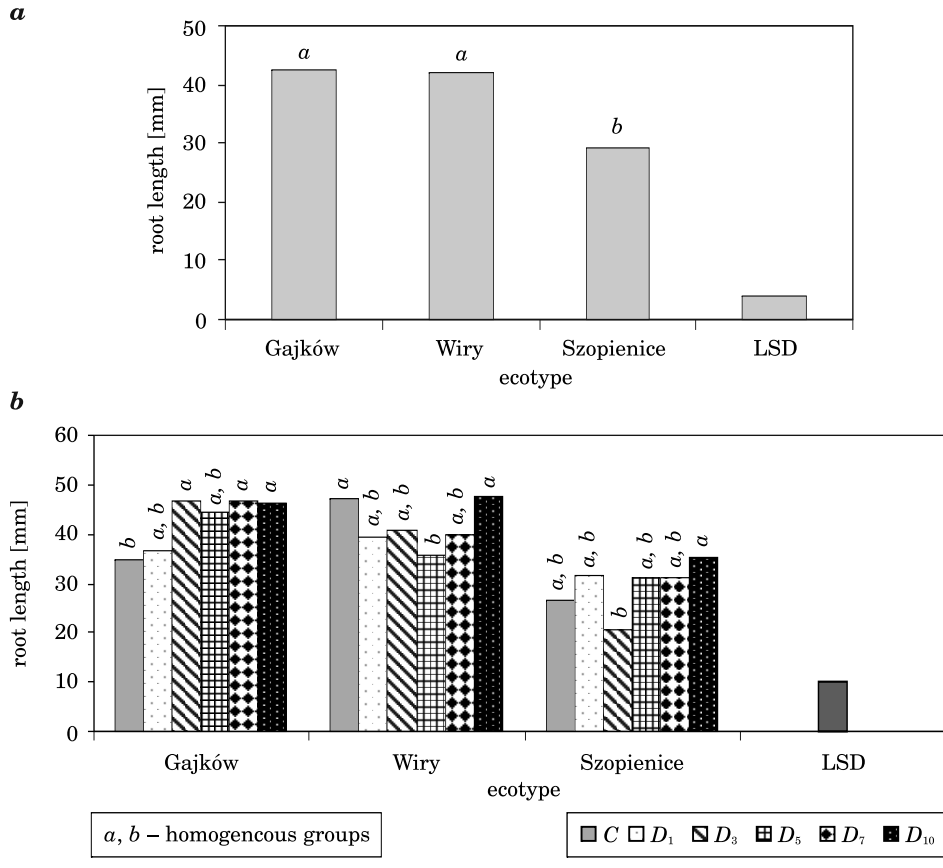

Fig. 4. Effect of stimulation of selected ecotypes of *Silene vulgaris* seeds on the embryonic root length: *a* – ecotype; *b* – interaction ecotype x dose; $LSD_{\alpha=0.05}$

Interaction proved the effect of stimulation in Gajków ecotype after application of three-, seven and tenfold irradiation (46.73; 46.73 and 46.17 mm respectively) in comparison to the length of embryonic root developed from control seeds (34.8 mm). Wiry ecotype responded by reducing the length of embryonic root after application of dose D_5 by 11.47 mm in relation to control.

The ecotype coming from Szopienice, as in the case of its germination capacity, did not respond to pre-sowing exposition of seeds to semiconductor laser beams (Figure 4). Analysis of variance, conducted for the length of above – ground parts of a seedling, proved significant diversity of laser irradiation doses, ecotypes, as well as interaction ecotype x dose. The examined *S. vulgaris* ecotypes significantly differed in the length of seedlings. The longest seedlings were produced by natural ecotype Gajków (9.03 mm), the second longest was Wiry ecotype (8.18 mm), while the shortest seedlings characterized the ecotype

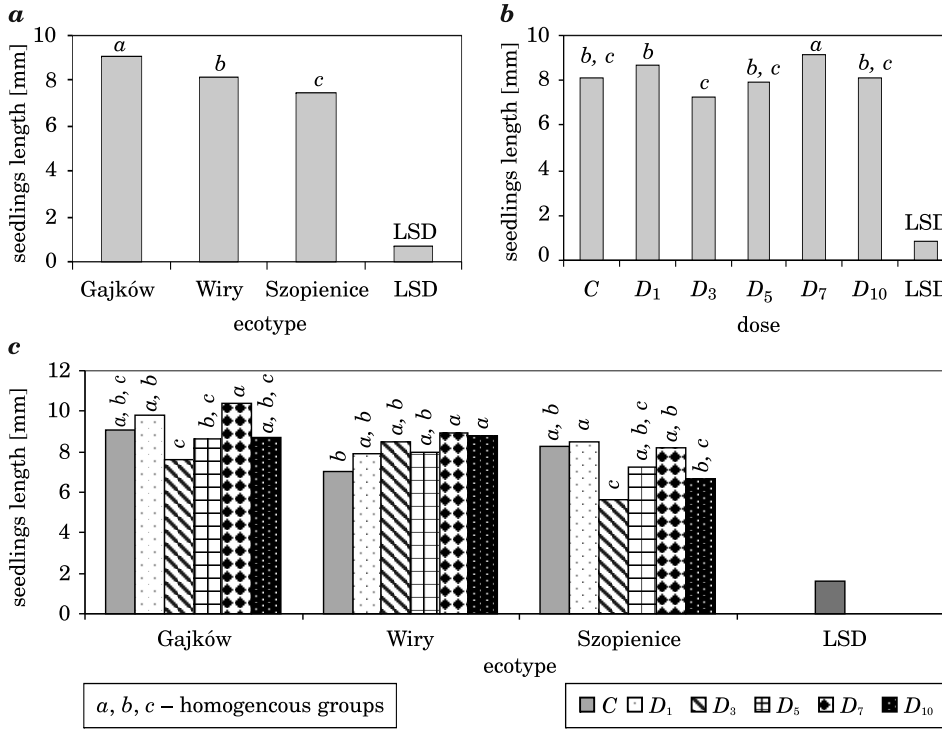

Fig. 5. Effect of stimulation of selected ecotypes of *Silene vulgaris* seeds on seedlings length: *a* – ecotype; *b* – dose; *c* – interaction ecotype x dose; $LSD_{\alpha=0.05}$

originating from Szopienice – 7.42 mm (Figure 5). Among the doses applied, only sevenfold irradiation of seeds resulted in the effect of stimulation, causing development of seedlings measuring 9.17 mm in length as compared to control seedlings which were 8.12 mm long (Figure 5). On the basis of interaction ecotype x dose it was possible to state the effect of stimulation in Wiry ecotype after introduction of doses D_7 and D_{10} (8.9 and 8.8 mm), while control produced seedlings 7.0 mm long. The ecotype from Szopienice showed reduction in above – ground part of seedlings by 2.7 mm and ecotype A did not show any response to pre-sowing irradiation (Figure 5). As far as the length of cotyledon was concerned, analysis of variance showed significant differences in laser irradiation doses, ecotypes and interaction ecotype x dose. The longest cotyledon featured Gajków ecotype (7.43 mm). Wiry ecotype produced cotyledon of 6.86 mm in length, while significantly shortest one was developed by Szopienice ecotype (6.27 mm) – Figure 6. Among diversified doses of laser radiation only sevenfold irradiation caused the response on the side of the examined ecotypes. Dose D_7 resulted in significant elongation of cotyledon – 8.49 mm in relation to cotyledons produced by control seedlings – 6.82 mm

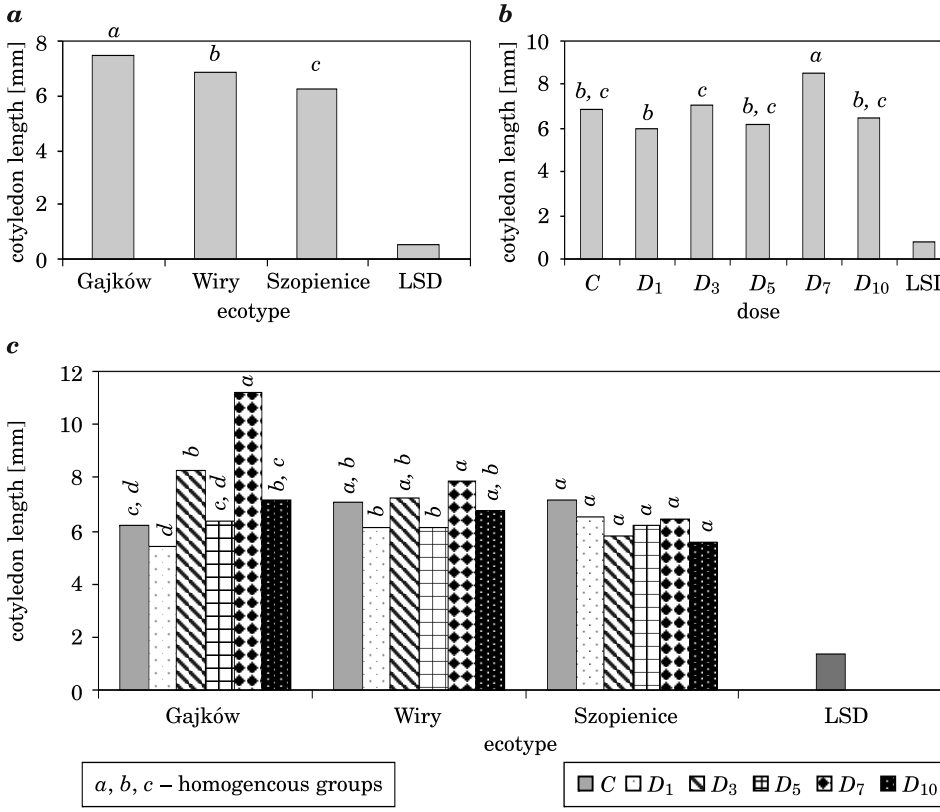

Fig. 6. Effect of stimulation of selected ecotypes of *Silene vulgaris* seeds on cotyledon length: *a* – ecotype; *b* – dose; *c* – interaction ecotype x dose; $LSD_{\alpha=0.05}$

(Figure 6). Considering three examined ecotypes, of *S. vulgaris* in terms of the length of cotyledons, only „Gajków" ecotype showed the effect of stimulation. After application of seven – and threefold irradiation cotyledons became elongated by 4.97 and 2.04 mm respectively in relation to control seedlings cotyledons (6.23 mm). The remaining ecotypes (Wiry and Szopienice), subjected to the research, did not show any response to the use of pre-sowing irradiation of seeds with semiconductor laser beams (Figure 6). In the studies, in selected genotypes of cereals, a significant increase in energy and germination after the introduction of pre-sowing laser irradiation was observed. Stimulation of morphological traits such as seedling root length of embryonic root, coleoptiles and aboveground parts of the seedlings were recorded after application of three- and five-time irradiation (SZAJSNER 2009). Research conducted by PODLEŚNY (2000, 2002) and PODLEŚNY et al. (2012) showed

positive effects of pre-sowing laser stimulation on the growth and development of lupine and faba bean seedlings. Similar effects were obtained by SZAJSNER et al. (2013) and PROŚBA-BIAŁCZYK et al. (2013) in studies on the effects of laser radiation on the sugar beet seeds.

Research by ŚLIWKA and JAKUBIAK (2009, 2010) on laser stimulation of plants, confirmed statistically significant increase in duckweed and yellow iris biomass in the first and in the subsequent years of conducting experiment, without any necessity of re-exposure of plant material to laser light. Stimulated plants characterized considerable resistance to decrease in temperature, as well as higher survival rate in subsequent years of the experiment. In varieties of energy willows there was also observed increased biomass of leaves after irradiation with laser diode (JAKUBIAK, ŚLIWKA 2008). Exposure to coherent light did significantly influence on increase in roots biomass, their length and density in hydroponic cultivation in salt solutions, which proves acceleration of the process of rhizogenesis (JAKUBIAK, ŚLIWKA 2009).

Conclusions

Conducted research and observation undertaken in the course of breeding of selected *Silene vulgaris* ecotypes allow to draw the following conclusions:

1. *Silene vulgaris* plants of serpentine dump (Wiry ecotype and Zn – Pb ore Szopienice ecotype) produce smaller seeds in comparison to seeds of Gajków natural ecotype.

2. Application of dose D_5 caused increase in germination capacity by 6.22%, while the dose D_7 resulted in the elongation of the aboveground parts of the seedlings, as well as significant increase in the cotyledons in the studied ecotype.

3. After seeds irradiation with doses D_3, D_7 and D_{10}, embryonic root elongation in natural Gajków ecotype was recorded. Significantly longer cotyledons were observed in this ecotype under the influence of D_7 and D_3 doses.

4. Improved germination in Wiry ecotype was observed after application of doses D_1, D_3 and D_5, while under the influence of dose D_7 and D_{10} a significantly longer seedling was produced.

5. Pre-sowing application of laser radiation did not cause any effects in Szopienice ecotype.

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