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[1]Institute of Biology, Pedagogical University of Kraków, Podchorążych 2, 30-084 Kraków, Poland
[2]Limanowa Hospital, Piłsudzkiego 61, 34-600 Limanowa, Poland
[3]Institute of Cardiovascular and Medical Sciences, University of Glasgow, 126 University Place, Glasgow G12 8TA, United Kingdom
[4]Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Science, Beijing 100081, P.R. China
*e-mail: katarzyna.mozdzen@up.kakow.pl

BARTOSZ RÓŻANOWSKI[1], KATARZYNA MOŻDŻEŃ[1,*], JADWIGA WÓJCIK[2], RYSZARD NOSALSKI[3], PEIMAN ZANDI[4], YAOSHENG WANG[4]

Influence of He-Ne laser irradiation and cadmium and lead on changes in cell cycles at *Zea mays* L.

Wpływ napromieniowania laserem He-Ne oraz kadmu i ołowiu na zmiany w cyklach komórkowych *Zea mays* L.

Summary. The aim of the study was to investigate the effect of red radiation emitted by helium-neon laser (He-Ne) with a wavelength $\lambda = 632.8$ nm and trace elements: cadmium (Cd) and lead (Pb), as cadmium nitrate and lead nitrate solutions, at 30 ppm concentrations, on the cell cycle of root-tip meristem cells in maize. Red light stimulated the mitotic activity of the meristemic cells of the maize root-tip meristem growth after irradiation of seed samples with simultaneous treatment in the presence of Cd or Pb. The mitotic index was inhibited when the seeds were treated with distilled water with the addition of Cd or Pb salts. Seeds treated with laser had significantly the highest mitotic index. Results revealed that maize seed pre-treatment with the red light protects the root-tip meristem cells against the mitodepressive effect of Cd and Pb ions. Seed biomodulation using red light emitted by He-Ne laser can positively effect the germination and plant growth.

Key words: laser, mitotic index, plants, photobiomodulation, trace elements

INTRODUCTION

Plants in the natural environment are exposed to various stress factors, thus to minimize the negative impact of different unfavourable environmental factors on plants, including trace elements, various methods may be used, including photobiomodulation using low-level laser light. Many experiments have shown a number of possibilities to use this innovative method for some pro-ecological activities [Dobrowolski et al. 1987, Dobrowolski and Różanowski 1998, Różanowski 1999, Dobrowolski et al. 2017]. Laser stimulation can increase tolerance of plant material to adverse the environmental conditions. For example, it increases germination energy and accelerates growth of some species [Pietruszewski et al. 2007, Mosneaga et al. 2018], tolerance to osmotic stress [Qiu et al. 2010], high soil salinity [Li et al. 2016, Qiu et al. 2018], high concentration of cadmium in the environment [Qiu et al. 2013], drought [Metwally et al. 2014, Qiu et al. 2017] or frost [Truchliński et al. 2002].

Maize (*Zea mays* L.) is a very valuable plant; it is used in over 300 modern industries. It is usually used for the production of bioethanol, biodiesel, biogas (methane), animal food, corn sweeteners, edible oil and starch, for food and industrial purposes. The global annual production of maize seeds is around 1030 million tons. In 2016, total maize production in the European Union amounted to 60.3 million tons [Wojcieszak et al. 2018]. Almost one hundred years of research, maize has become one of the most important plant species due to its intriguing and dynamic genome and the economic importance of crops. The size of its nuclear genome and its cultivars range from 4.62 to 6.29 pg [Silva et al. 2018]. It is a one-year diploid species, it has an average genome size of 2n = 20 chromosomes that contain about 59 thousand genes [Alberts et al. 2019].

Demand for ecological products growing year by year and contributes to the search for innovative and environmentally safe methods. Therefore, it was hypothesized that the red radiation emitted by the He-Ne laser can increase the resistance of germinated maize seeds to the presence of toxic trace elements, i.e. Cd or Pb in cadmium nitrate and lead nitrate solutions at 30 ppm concentrations. For this aim, the influence of red radiation and trace elements Cd and Pb on the life cycle of meristematic cells of root-tips was examined by determining the value of the mitotic index (Im) (1), and the correct functioning of the karyokinetic spindle (2) in the *Z. mays* seeds.

MATERIAL AND METHODOLOGY

Photobiomodulation of seeds with helium-neon laser

Maize seeds (*Zea mays* L.) were irradiated with a helium-neone (He-Ne) laser with a wavelength λ = 632.8 nm, a surface radiation power density of 2 W m^{-2} and an output radiation power of 18 mW. A laser beam diffusion system with a diameter of 8 cm was used, the exposure time was 90 seconds, corresponding to a surface energy density of 1×180 J m^{-2}.

Analysis of the mitotic index

For the experiment 300 maize seeds were used, from which six experimental groups were created. Each sample consisted of 50 seeds and such samples were used in the following treatments: (1) distilled water – control sample, (2) seeds irradiated with helium and neon laser (He-Ne) for 90 s – N90, (3) seeds treated with lead nitrate of 30 ppm concentration – Pb, (4) seeds irradiated with He-Ne laser for 90 s and treated with lead nitrate of 30 ppm concentration – N90 + Pb, (5) seeds treated with cadmium nitrate of 30 ppm concentration – Cd, (6) seeds irradiated with He-Ne laser for 90 s and then treated with cadmium nitrate of 30 ppm concentration – N90 + Cd.

The maize seeds immediately after irradiation for 90 s were placed on Petri dishes (Ø 9 cm) with a filter paper soaked in the distilled water or the lead and cadmium nitrate solutions depending on the experimental group. The seeds were placed in a growth chamber (Angelantoni Industrie, Italy) at temperature 20 ±1°C, photoperiod 12/12 h (day/night), light intensity 200 μmol m^{-2} s^{-2}, and relative humidity RH 60–70%.

After five days at the peak of the mitotic activity of meristematic cells, between 12 and 14 h, the roots-tips were taken for further studied. They were fixed in Carnoy's solution and then on a cytochemical Feulgen's reaction was performed [Feulgen and Rosenbeck 1942]. Microscopic slides were made, which were analyzed under a biological microscope (Hund H-600), under a 600-fold magnification.

To find differences in the mitotic activity between the control material and experimental groups, the mitotic indexes (Im) were calculated according to the formula:

$$Im = (\text{number of divisible cells} / \text{total number of cells}) \times 100\%$$

In order to determine the proper functioning of the kariokinetic spindle in the studied groups, the D'Amato ratio was calculated from the formula according to D'Amato [1948]:

$$\text{D'Amato ratio} = \text{number of metaphases} / (\text{number of anaphases} + \text{number of telephones})$$

Statistical analysis

In order to assess the statistical significance of cell cycle change, the analysis of variance (ANOVA) for single classification was applied under the influence of laser radiation on meristematic cells of root growth maize between the control group and experimental groups. Significance of differences between means were assessed using the Tukey test, at $p = 0.05$, using the Statistica version 13.0 program.

RESULTS AND DISCUSSION

On the basis of cytological tests, it can be concluded that the exposure of *Zea mays* seeds on the red laser light He-Ne for 90 s caused changes in the life cycle of the meristematic cells of the root-tips (Tab. 1, Fig. 1).

The average value of the mitotic index for the control material was 7.02%, in the group exposed by a helium and neon laser (N90) with a surface energy density of 180 J m^{-2} value was 9.07% which was a statistically significant increase compared to the control material (Fig. 1A). This result is consistent with the literature data that red light is a stimulator of cell division [Li et al. 2016]. This laser radiation induces the internal energy of seeds, which leads to increased germination through an increase in the activity of amylases and proteases involved in germination physiology, growth and development of seedlings [Muthusamy et al. 2012]. Laser energy initiates and enhances physiological and biochemical processes, causing the transformation of light energy into chemical energy [Hernandez et al. 2010]. As a consequence, the biochemical and physiological metabolism of plants is accelerating [Podleśny et al. 2012, Swathy et al. 2016]. Many of these changes are an adaptive response by which plants deal with various stress factors including those with toxic trace elements [Das et al. 1997]. The effect of exposure to the red light of seeds is also revealed in the later stages of development and growth. It also influences the increase in the intensity of photosynthesis and plant transpiration [Podleśny et al. 2012]. Researches confirm that laser light is a stimulator of mitotic activity, resulting in increased germination capacity, early emergence and higher biomass growth [Muthusamy et. al. 2012, Podleśna et al. 2015]. Penetration of light into the tissue depends on its structure, water content and the presence of photo-receptors and is individual for each species. Properly selected type of light, the wavelength, the energy and power, type of exposure (continuous, intermittent), as well as the duration of the laser's operation on a specific tissue (cell) may affect not only the growth of plants, but also on the ability to accumulate trace elements [Różanowski 1999]. The use of lasers is safe and effective in fighting the environmental pollution [Dobrowolski et al. 2017].

Table 1. The number of cells from the individual phases of the cell cycle maize seeds

Groups	Phases of the cell cycle				
	interphase	prophase	metaphase	anaphase	telophase
Control	1830.33^{b} ±5.21	97.33^{c} ±2.89	12.33^{d} ±2.52	13.67^{c} ±7.57	14.67^{b} ±1.15
N90	2455.00^{a} ±5.80	160.00^{a} ±3.54	31.67^{a} ±1.54	23.33^{a} ±1.50	30.67^{a} ±1.61
Pb	1921.67^{b} ±10.60	86.00^{d} ±7.94	15.00^{c} ±6.56	12.67^{cd} ±3.21	5.67^{c} ±0.58
Cd	2343.33^{a} ±7.04	78.33^{e} ±3.51	18.33^{b} ±3.21	10.00^{d} ±3.61	17.00^{b} ±3.06
N90 + Pb	2105.00^{ab} ±8.01	123.67^{b} ±4.04	19.67^{b} ±4.16	15.33^{b} ±1.53	12.33^{bc} ±1.96
N90 + Cd	2879.67^{a} ±7.59	127.00^{b} ±2.85	33.33^{a} ±5.51	12.33^{cd} ±2.31	33.67^{a} ±1.04

Values (±SD) n = 5 marked with different letters in the column differ significantly according to the Tukey test, p = 0.05; Control – seeds watered with distilled water, N90 – seeds irradiated with helium and neon laser (He-Ne) for 90 s, Pb – seeds treated with lead nitrate of 30 ppm concentration, Cd – seeds treated with cadmium nitrate of 30 ppm concentration, N90 + Pb – seeds irradiated with He-Ne laser for 90 s and treated with lead nitrate of 30 ppm concentration, N90 + Cd – seeds irradiated with He-Ne laser for 90 s and then treated with cadmium nitrate of 30 ppm concentration

A significant decrease in the value of the mitotic index in the material treated with Cd or Pb indicates the inhibition of their effect on the life cycle of the cell. As compared to the control group, a slight decrease in the value of the mitotic index was observed in groups where the seeds were first exposed with red light and then treated Cd or Pb solutions at 30 ppm concentration (Fig. 1A). However, the lack of clear differences in the values of the D'Amato coefficient indicates that the radiation, Cd and Pb in the concentration used do not disturb the significant functioning of the karyokinetic spindle (Tab. 1, Fig. 1B). Previous research confirms that He-Ne laser radiation can counteract oxidative damage and have a protective effect against stress factors [Li et al. 2016].

The plants have developed different adaptations to counteract the adverse influence of abiotic factors. These responses are strictly controlled both at the level of cells, tissues and metabolic processes through comaratively complex regulatory pathways that are still not fully elucidated [Li et al. 2016]. For example, particular plant species use different mechanisms of protection against the penetration of trace elements. Some produce tolerance mechanisms to avoid their penetration into plants. The roots form the first barrier against trace elements [Wierzbicka 1998, Ogundipe and Babarinde 2017]. In addition, there are processes of immobilization of heavy metals in plants, e.g. precipitation of compounds in the form of ortho- and pyrophosphates or in another crystalline form on the cell membranes of roots, stems and leaves [Kabata-Pendias 2010].

The plants take trace elements from the soil, generally in proportion to the increase in the concentration of these components in soil. This process is regulated by many factors, among which the decisive are soil pH, organic matter content, bacterial flora activity, redox potential and soil aeration [Kabata-Pendias 2010]. In order to increase the accumulation potential of plants, chelating substances may be used, which release metals from soil particles and form soluble complexes with them [Luo et al. 2006], and at the same time do not affect the reduction of plant biomass and bacterial growth. The choice of maize for phytoremediation purposes may also be justified by the strongly developed, in the upper soil layers, the root system and its accumulation capacity [Trachsel et al. 2011].

Biostimulated maize can be grown not only for consumption, but also for the purification of the environment and for energy purposes (biofuels, biogas). Therefore, further studies on the impact of laser biostimulation on plant life processes may contribute not only to increasing production and improving food quality, but also to effective protection and shaping of the natural environment, especially in industrial contaminated regions [Dobrowolski et al. 1987, Dobrowolski and Różanowski 1998, Różanowski 1999, Dobrowolski et al. 2017]. The positive effect of radiation on germination of maize seeds is manifested by an increase in the value of the mitotic index and proper functioning of the karyokinetic spindle in the presence of toxic trace elements (Fig. 1, Tab. 1).

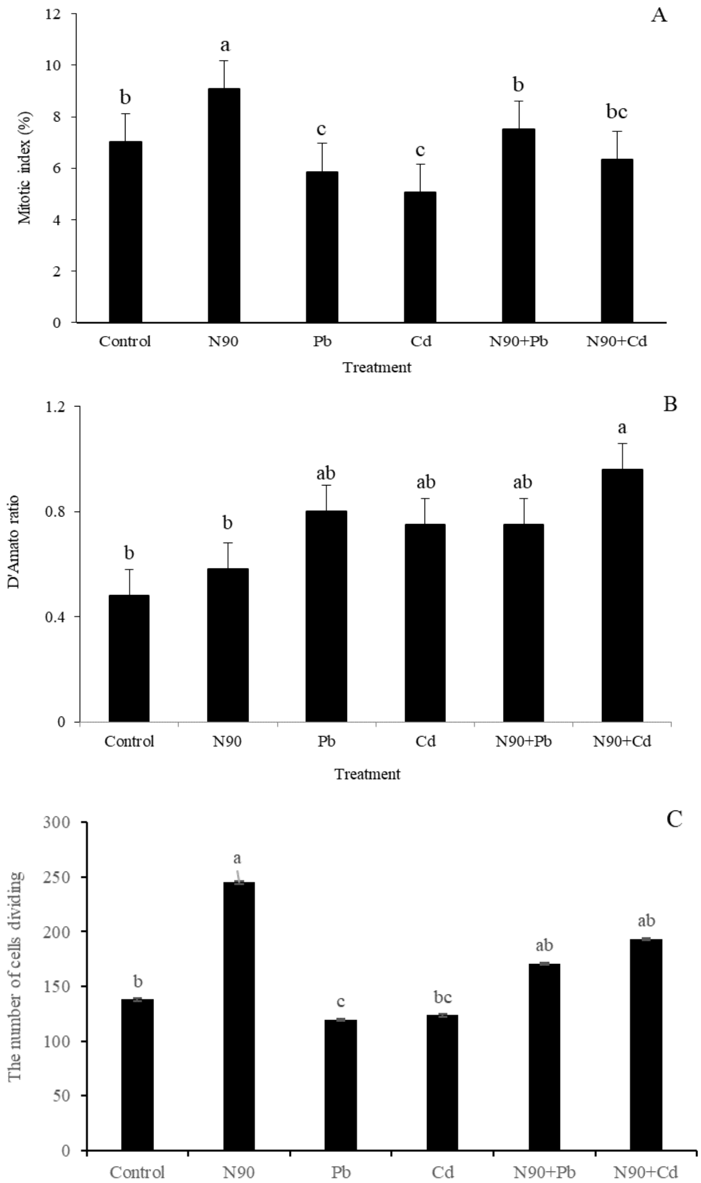

Fig. 1. The values of mitotic index (A) and D'Amato ratio (B) indexes, and the number of cells dividing (C) of *Zea mays* L. seeds: Control – seeds watered with distilled water, N90 – seeds irradiated with helium and neon laser (He-Ne) for 90 s, Pb – seeds treated with lead nitrate of 30 ppm concentration, Cd – seeds treated with cadmium nitrate of 30 ppm concentration, N90 + Pb – seeds irradiated with He-Ne laser for 90 s and treated with lead nitrate of 30 ppm concentration, N90 + Cd – seeds irradiated with He-Ne laser for 90 s and then treated with cadmium nitrate of 30 ppm concentration; values (±SD) n = 5 marked with different letters differ significantly according to the Tukey test, p = 0.05

The environmental pollution cannot be limited if it will be frequently used chemical additives that are harmful to biological systems. Clean, environmentally friendly, more efficient and cheaper techniques are a viable alternative, and laser pre-sowing seed treatment is very interesting because is non-destructive, safer and cheap.

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Streszczenie. Celem badań było określenie wpływu promieniowania czerwonego emitowanego przez laser helowo-neonowy (He-Ne) o długości fali λ = 632,8 nm oraz pierwiastków śladowych: kadmu (Cd) i ołowiu (Pb), w wodnych roztworach azotanu kadmu i azotanu ołowiu, w stężeniu 30 ppm, na cykl życia komórek merystemów korzeniowych kukurydzy (*Zea mays* L.). Wykazano, że światło czerwone stymuluje mitotyczną aktywność komórek merystematycznych korzeni *Z. mays* po napromieniowaniu nasion, nawet w obecności Cd lub Pb. Nasiona traktowane laserem miały znacząco najwyższy wskaźnik mitotyczny. Wartości wskaźnika mitotycznego zostały zahamowane, gdy nasiona traktowano roztworami z Cd lub Pb. Wyniki wskazują, że wstępne traktowanie nasion kukurydzy światłem czerwonym chroni komórki merystemu korzeni przed niekorzystnym oddziaływaniem jonów Cd i Pb. Biomodulacja nasion za pomocą światła czerwonego emitowanego przez laser He-Ne może pozytywnie wpływać na kiełkowanie i wzrost roślin.

Słowa kluczowe: laser, wskaźnik mitotyczny, rośliny, fotobiomodulacja, pierwiastki śladowe

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