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EFFECT OF LASER IRRADIATION ON SEED GERMINATION AND ROOT YIELD OF SCORZONERA (*SCORZONERA HISPANICA* L.)

Marcela Krawiec[1], *Agata Dziwulska-Hunek*[2], *Salwina Palonka*[1], *Magdalena Kapłan*[1], *Piotr Baryła*[1]

[1]Department of Horticultural Nursery and Seed Production, University of Life Sciences in Lublin
Leszczyńskiego 58, 20-680 Lublin, Poland
[2]Department of Physics, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland
e-mail: marcela.krawiec@up.lublin.pl

A b s t r a c t. The problem in the cultivation of scorzonera is often poor, slow and non-uniform field emergence despite the use of seeds with high germination capacity for the sowing. The goal of the study was to determine the effect of pre-sowing laser stimulation of seeds on the germination, emergence and root yield of scorzonera. Seeds were irradiated with a He-Ne laser with wavelength of 632.4 nm. Two doses of laser beam irradiation were applied in terms of surface power density – 6 and 8 mW cm^{-2}. Seeds were irradiated 1, 3 and 5 times, and the duration of each irradiation treatment was *ca*. 0.1 s. Seed treatment with laser light caused an increase of germination capacity, radicle length and dry weight of seedling, and an improvement of field emergence and only a partial increase of the total yield of roots. Depending on the irradiation dose applied, the germination capacity of the seeds increased by 1.5-13.2% in relation to the control. The most beneficial effect on the germination capacity and field emergence was that of the 5-time laser irradiation. Increase of total root yield as a result of pre-sowing seed irradiation was related with increased emergence.
K e y w o r d s: emergence, germination capacity, He-Ne laser light, seedlings

INTRODUCTION

Scorzonera (*Scorzonera hispanica* L.) is a root vegetable. The high health-promoting value of the species is determined by its content of vitamins B, E, C and of easily assimilable mineral components (Mg, P, Fe, K, Na). Scorzonera is one of the best sources of inulin. It is a natural prebiotic stimulating the growth of beneficial intestinal flora that improves the functioning of the gastrointestinal tract (Nowak *et al.* 2012). Apart from that, inulin – due to its low calorific value and hypoglycaemic effect – finds an application in the diet of diabetes patients (Niness 1999).

The popularisation of scorzonera cultivation encounters various barriers, e.g. the difficult and costly harvest of roots which are long and fragile, and thus prone to mechanical damage (Nuez and Bermejo 1994). Field emergence is often poor, slow and non-uniform, despite the use for sowing seeds of high germination capacity, especially under conditions of water deficiency in the soil. Consequence of poor emergence is low plant density per unit area and unsatisfactory yield of roots. It should also be emphasised that scorzonera seeds rapidly lose germination capacity (Stephens 1994). Therefore, it is extremely important to search for effective methods of improvement of seed germination, seedlings emergence and yield of scorzonera.

Physical methods, including laser radiation, are among those methods of sowing material improvement that are safe for the environment (Koper 1994). Studies conducted at various research centres indicate a positive influence of seed irradiation on the processes of growth and development of crop plants, mainly cereals, legumes and certain vegetables. Pre-sowing laser stimulation can cause an improvement of germination and emergence, an increase of yields or a shortening of the period of vegetation, and even enhance the resistance of plants to environmental stresses (Szyrmer and Klimont 1999, Wilczek *et al.* 2005, Podleśny *et al.* 2012, Prośba-Białczyk *et al.* 2013, Krawiec *et al*. 2015). However, the available literature does not provide comprehensive studies documenting the effect of that factor on both seed germination and emergence and on the yields of root vegetables.

Many authors prove that the success of laser stimulation is dependent on wavelength, irradiation time and irradiation dose (Rybiński 2000, Hernandez *et al.* 2006, 2010, Gładyszewska 2011). Generally, seed stimulation is done with the use of small doses of laser radiation. Lower doses of laser activate plants, resulting in increasing bioenergetical potential of the cells and higher activation of their biochemical and physiological processes (Rybiński 2000). Seed stimulation is performed most frequently with the use of low power lasers and relatively long exposure times, measured in seconds or minutes (Koper 1994). Some researchers are of the opinion that seeds should be irradiated several times with small doses of radiation to eliminate the possibility of mutation (Inyushin *et al*. 1981). Higher doses influence the genetic material of the cell, leading to genetic changes of plant traits (Rybiński 2000).

The objective of the study was to determine the effect of pre-sowing laser stimulation, with the use of several-time exposure to small doses of radiation, on the germination, emergence and root yield of scorzonera.

MATERIAL AND METHODS

The study was conducted in the years 2012-2013 at the University of Life Sciences in Lublin. The experimental material was seeds of scorzonera cv.

'Meres'. The germination capacity of the seeds was 77.5 and 76.3% in 2012 and 2013, respectively. The irradiation was performed with the use of an apparatus for pre-sowing stimulation of seeds with a divergent beam, designed by Dygdała and Koper (1993). The seeds were irradiated with a He-Ne laser with wavelength of 632.4 nm. The first experimental factor was the surface power density of the divergent laser beam, of 0, 6 and 8 mW cm^{-2}. The second factor was the number of irradiation treatments – 1, 3 and 5. The duration of each irradiation of seeds in free fall was ca. 0.1 s. Non-stimulated seeds were the control treatment. The combinations obtained were designated as follows: R0 (control), R6x1, R6x3, R6x5, R8x1, R8x3, R8x5.

In the laboratory experiment the following parameters were estimated after seed stimulation with laser light: germination capacity, share of abnormal seedlings, hypocotyl and radicle length, dry weight of seedling. The assessment of the selected germination parameters was conducted under the standard germination test done in 4 repetitions on 50 of seeds from each experimental setting. Seeds were sown on moistened double blotting paper on the day following laser stimulation. Then, they were rolled up and placed upright in containers. The containers were covered with plastic bags. The seeds germinated in a thermostat at temperature of 20°C (±1°C), in darkness. The germination capacity (expressed in terms of the rate of normally germinating seeds) and share of abnormal seedlings were calculated after 8 days from sowing the seeds on blotting paper (ISTA 2012).

Based on the seedling growth test, the hypocotyl and the radicle lengths and the dry seedling weight were estimated. The seedling growth test was conducted in 4 replications of 25 seeds. The germination conditions were the same as described previously. After 8 days the length of hypocotyl and radicle of normal seedling were determined. Normal seedlings from each replication were dried at temperature of 80°C for 24 hours. In that manner, the dry weight of a single seedling for each treatment was calculated (Hampton and TeKrony 1995).

The field experiment was done by using a randomised complete block design in 4 repetitions of 100 seeds. The field experiment was conducted at the Felin Experimental Farm of the University of Life Sciences in Lublin (51°23′N, 22°56′E) on a grey-brown podzolic soil developed from loess formations overlying chalk marls, with a grain size composition corresponding to that of weak silty loams. Seeds were sown on the 24th of April, 2012, and on the 23rd of April, 2013. Each 100 seeds were sown in two rows 2.5 m long, spaced 0.40 m apart. Upon the appearance of the first seedlings, daily counting of the seedlings was done. The counting was continued until the 3rd day after the day when no new seedlings appeared. Based on those measurements, the emergence and the mean time of emergence were calculated. The mean emergence time was expressed by Pieper's coefficient (W). It was determined using the following model:

$$W = \Sigma(d \times pd)/k \text{ (days)} \quad (1)$$

where : d – day of seeds emergence, pd – number of seedlings emerged on given day, k – total number of emerged seedlings.

The roots of scorzonera were harvested at the beginning of October each year. The total yield of roots and the mean weight of root were estimated.

The two years of the study differed strongly in terms of the amount of precipitation. The monthly rainfalls in 2012 were considerably below the multi-year average. Whereas, the sums of precipitations in 2013, in all the months of the study (with the exception of August), considerably exceeded the multi-year mean. The diverse moisture conditions in the two years could have an effect on the emergence and on the yield of scorzonera. Air temperatures during the April-September study periods in both years were higher by 0.7-2.9°C than the mean multi-year temperatures (Tab. 1).

Table 1. Mean monthly air temperatures and precipitation in the years 2012-2013 against the multi-year mean values (1951-2005) at AES Felin near Lublin

Month	Temperature (°C)			Sums of precipitation (mm)		
	2012	2013	Multi-year mean	2012	2013	Multi-year mean
April	9.2	8.1	7.4	39.1	51.1	40.2
May	14.6	15.3	13.0	33.8	101.6	57.7
June	16.8	18.5	16.2	67.6	105.9	65.7
July	20.7	19.2	17.8	61.2	126.1	83.5
August	18.5	19.2	17.1	44.7	17.8	68.6
September	14.4	11.8	12.6	38.4	64.6	51.6

The results obtained were processed statistically using the analysis of variance ANOVA (STATISTICA 6.0). Intervals of confidence were determined with the Tukey test at the level 0.05.

RESULTS AND DISCUSSION

The study presented herein demonstrated that laser treatment of scorzonera seeds had a beneficial effect on their germination capacity (Tab. 2). It is very important because that parameter is the principal and internationally accepted criterion for seed viability (Hampton and TeKrony 1995). Depending on the irradiation dose applied, germination capacity increased by 1.8-10.5% (2012) and 1.5-13.2% (2013) in relation to the untreated control. In the presented study, seed germination capacity depended on the number of irradiation treatments. It was found that with increasing number of irradiations, the germination capacity of the seeds increased and the share of abnormal seedlings decreased. The greatest germination capacity and the lowest number of abnormal seedlings were noted after 5-time irradiation of seeds with both surface power density values (Tab. 2). These results support the research by Wilczek *et al.* (2005) showing a positive effect of increasing numbers of irradiations on the germination capacity of alfalfa seeds. A study by Szyrmer and Klimont (1999) demonstrated that laser stimulation caused an improvement of quality of French bean seeds, reducing the number of seeds germinating abnormally. Authors of studies concerning seed irradiation indicate that seed germination depended on the dose applied, the plant species and even the cultivar (Rybiński 2000, Klimont 2001, 2002, Hernandez *et al.* 2010, Gładyszewska 2011). Research by Krawiec *et al.* (2015) concerning the effect of continuous time of scorzonera seed irradiation (1-30 minutes) showed an increase of germination energy and capacity as well as an acceleration of seed germination. The response of seeds to stimulation depended on the lot of seeds of the cultivar 'Maxima'. Seed of low quality displayed a greater increase of germination capacity compared to seeds of better quality.

Table 2. Effect of laser irradiation on germination of scorzonera seeds

Irradiation dose	Germination capacity (%)		Abnormal seedlings (%)	
	2012	2013	2012	2013
R0	77.5 ^{b}*	76.3 ^{c}	18.5 ^{c}	18.7 ^{c}
R1×6	79.3 ^{ab}	78.0 ^{bc}	15.7 ^{bc}	17.0 ^{bc}
R3×6	81.3 ^{ab}	78.8 ^{bc}	13.7 ^{bc}	14.7 ^{bc}
R5×6	88.0 ^{a}	85.3 ^{ab}	5.3 ^{a}	11.7 ^{ab}
R1×8	79.5 ^{ab}	77.8 ^{bc}	15.0 ^{bc}	16.7 ^{bc}
R3×8	84.0 ^{ab}	81.5 ^{b}	11.0 ^{ab}	12.0 ^{b}
R5×8	86.0 ^{a}	89.5 ^{a}	9.5 ^{ab}	6.5 ^{a}

*Means with different letters in the same column are statistically different at p = 0.05

The mean hypocotyl length of seedlings grown from the control seeds was 2.62 cm in 2012 and 2.51 cm in 2013 (Tab. 3). No effect of pre-sowing stimulation of scorzonera seeds on seedling hypocotyl elongation was demonstrated. These results are not in conformance with research by other authors that show a positive effect of seed irradiation on seedling growth. Podleśny *et al.* (2012), as a result of irradiation, observed an elongation of the hypocotyl in white lupine and faba bean, while Drozd and Szajsner (2007) noted hypocotyl elongation in cucumber. The varied response to laser radiation may be related with the different structures of seedlings of particular species. The aboveground part of scorzonera seedling (hypocotyl) is short as the plant produces a shortened stem and a rosette of leaves.

In our studies, seed stimulation with all of the doses of laser light caused an elongation of the radicle. In 2012, 1- and 5-time irradiation with laser of 6 mW cm^{-2} power and 5-time irradiation with laser of 8 mW cm^{-2} power caused an increase of seedling radicle length relative to the control (Tab. 3). Whereas, in 2013, seed stimulation with laser of 8 mW cm^{-2} power caused an elongation of the radicle. These results confirm the research of other authors, indicating that laser radiation causes an elongation of roots of crop plants (Drozd and Szajsner 2007, Podleśny *et al.* 2012).

In 2012, as a result of seeds irradiation with all of the doses, an increase of seedling dry weight relative to the control was observed (Tab. 3). It was 13.0-16.0%. In 2013 an increase of seedling dry weight was noted as a result of 1- and 5-time laser stimulation at both power levels. The dry weight increase amounted to 7.3-20.9% relative to the control. These results are in accordance with the studies that show a beneficial effect of laser stimulation on increase of dry weight seedlings of maize (Hernandez *et al.* 2006), white lupine and faba bean (Podleśny *et al.* 2012), French bean and pea (Klimont 2001), and cucumber and tomato (Klimont 2002).

Table 3. Effect of laser irradiation of scorzonera seeds on hypocotyl length, radicle length and seedling dry weight

Irradiation dose	Hypocotyl length (cm)		Radicle length (cm)		Seedling dry weight (mg)	
	2012	2013	2012	2013	2012	2013
R0	2.62 ^{a}*	2.51 ^{a}	4.49 ^{b}	4.80 ^{b}	2.31 ^{b}	2.20 ^{b}
R1×6	2.71 ^{a}	2.57 ^{a}	5.29 ^{a}	5.53 ^{ab}	2.68 ^{a}	2.53 ^{a}
R3×6	2.75 ^{a}	2.44 ^{a}	4.88 ^{ab}	5.35 ^{ab}	2.66 ^{a}	2.41 ^{ab}
R5×6	2.69 ^{a}	2.36 ^{a}	5.09 ^{a}	5.40 ^{ab}	2.61 ^{a}	2.50 ^{a}
R1×8	2.51 ^{a}	2.52 ^{a}	4.57 ^{b}	5.92 ^{a}	2.69 ^{a}	2.66 ^{a}
R3×8	2.65 ^{a}	2.43 ^{a}	4.58 ^{b}	5.95 ^{a}	2.63 ^{a}	2.36 ^{ab}
R5×8	2.60 ^{a}	2.50 ^{a}	5.27 ^{a}	5.91 ^{a}	2.67 ^{a}	2.58 ^{a}

*Explanations as in Table 2

The increase of scorzonera seedling radicle length and dry weight noted in this study as a result of laser stimulation can be attributed to faster water uptake by irradiated seeds and to more advanced metabolic processes. A study conducted by Podleśny *et al.* (2012) demonstrated that irradiation had an influence on the mass of faba bean and white lupine imbibing seeds. The irradiated seeds enlarged their masses more quickly during imbibition in comparison to non-irradiated ones. The results obtained by those authors show that in irradiated seeds there was also an increase in the content of amylolytic enzymes which are responsible for the reactions of hydrolysis of polysaccharides. Compounds formed as a result of that hydrolysis are then used for the building of tissues of a new seedling.

In spite of the similar levels of germination capacity of the analysed seed lots in the two years of the study, differences were noted with regard to the field emergence (Tab. 4). That differentiation between the years was probably due to the different moisture conditions during the period of emergence. At the turn of April and May, 2012, a rainfall deficit was noted after the sowing of seeds, which could have inhibited seed germination and emergence. In 2013, after seed sowing, rainfall was abundant and notably exceeded the multi-year mean sums for April and May. At that time, more numerous and faster emergence was noted. In the experiment, the emergence of scorzonera seedlings obtained from untreated seeds amounted to 38.0 and 43.8% in 2012 and 2013, respectively. In 2012, no effect of pre-sowing stimulation of scorzonera seeds on field emergence was demonstrated. In 2013, seed irradiation with all the doses of laser light had a positive influence on seedling emergence. Depending on the dose applied, that increase was 4.9-14.2% relative to the control (Tab. 4). Despite the positive tendency, no effect of seed irradiation on the shortening of the emergence mean time was demonstrated. A beneficial effect of pre-sowing laser stimulation of seeds resulting in an increase and acceleration of plant emergence was noted with relation to other species (Klimont 2002, Podleśny and Podleśna 2004, Hernandez *et al.* 2006). The scale of the effects obtained varied and depended on the plant species and cultivar, and on the atmospheric conditions in the years of the studies. Hernandez *et al.* (2006) demonstrated that field emergence of maize depended on the dose of irradiation as expressed by laser power and time of exposure.

Table 4. Effect of laser irradiation of scorzonera seeds on seedlings emergence

Irradiation dose	Field emergence (%)		Mean emergence time (days)	
	2012	2013	2012	2013
R0	38.0 [a]*	43.8 [b]	15.19 [a]	11.12 [a]
R1×6	42.1 [a]	51.5 [a]	14.73 [a]	10.65 [a]
R3×6	41.4 [a]	53.3 [a]	14.93 [a]	10.86 [a]
R5×6	42.8 [a]	55.5 [a]	15.05 [a]	10.67 [a]
R1×8	42.4 [a]	53.3 [a]	14.89 [a]	10.98 [a]
R3×8	43.2 [a]	48.7 [ab]	14.75 [a]	10.92 [a]
R5×8	46.9 [a]	58.0 [a]	14.82 [a]	11.05 [a]

*Explanations as in Table 2

Differences were observed in the root yield of scorzonera in the particular years of the study (Tab. 5). In 2013, a year characterised by favourable moisture conditions for the cultivation of plants, a higher root yield was obtained than in 2012. The total root yield obtained from non-irradiated seeds in the years 2012 and 2013 was 20.6 and 24.5 t ha^{-1}, respectively. In 2013, a beneficial influence of laser exposure on the root yield was observed. The yield increased, depending on the laser light dose applied, by 1.2-13.5% relative to the control. In that year significantly higher root yield, compared to the control, was obtained from seeds treated 5 times with laser light of surface power density of 6 and 8 mW cm^{-2}. In 2012, a positive trend of increase in root yield as a result of stimulation was only observed, however, statistical analysis showed no difference compared to the control. In that year, the yield obtained from laser treated seeds increased, depending on the dose applied, by 2.9-10.7% relative to the control.

The results obtained confirm the research by other authors showing a positive effect of pre-sowing laser stimulation on the yields of crop plants. Among other things, seed irradiation caused an increase in seed yield of white lupine and faba bean (Podleśny and Podleśna 2004), in seed yield of pea (Podleśna *et al.*2015), in yield of green and dry matter of alfalfa (Ćwintal and Dziwulska-Hunek 2013), tomato fruits (Koper 1994, Vasilevsky and Bosev 1997), cucumber (Klimont 2002), peppers and onion (Vasilevsky and Bosev 1997). Among the root crops, pre-sowing laser light treatment caused an increase of root yield of sugar beet (Prośba-Białczyk *et al.* 2013).

Under the conditions of the field experiment conducted in the years 2012 and 2013, the mean weight of scorzonera root obtained from non-irradiated seeds was 59.6 and 69.5 g, respectively (Tab. 5). In both years, no effect of pre-sowing stimulation of scorzonera seeds on root weight was noted. However, the mean weight of root grown from the control seeds was bigger in comparison with the weight of roots produced from irradiated seeds. It appears that this can be related with lower plant density in the treatments without laser stimulation than in those with seed irradiation. It can be supposed that the weight of scorzonera root obtained in 2013 was greater because of the better moisture conditions in that vegetation season.

Table 5. Effect of pre-sowing laser irradiation of seeds on yielding of scorzonera roots

Irradiation dose	Total yield (t ha^{-1})		Root weight (g)	
	2012	2013	2012	2013
R0	20.6 ^{a*}	24.5 ^{b}	59.6 ^{a}	69.5 ^{a}
R1×6	21.5 ^{a}	26.8 ^{ab}	57.3 ^{a}	64.0 ^{a}
R3×6	21.2 ^{a}	26.3 ^{ab}	56.8 ^{a}	62.4 ^{a}
R5×6	22.8 ^{a}	27.5 ^{a}	56.3 ^{a}	61.8 ^{a}
R1×8	22.1 ^{a}	26.9 ^{ab}	57.9 ^{a}	62.7 ^{a}
R3×8	22.6 ^{a}	24.8 ^{b}	58.2 ^{a}	64.2 ^{a}
R5×8	22.3 ^{a}	27.8 ^{a}	57.5 ^{a}	63.5 ^{a}

*Explanations as in Table 2

Studies conducted by other authors indicated that yield increase as a result of pre-sowing seed irradiation can be an effect of increased number and mass of fruits (Klimont 2002), greater number of pods per plant (Klimont 2001, Podleśny and Podleśna 2004), or lower losses of plants from unit area in the course of the vegetation period (Podleśny and Podleśna 2004). In the study presented here, the increase of root yields of scorzonera can be a result of the higher emergence caused by the pre-sowing irradiation of seeds. Analysis of root yield revealed that the laser stimulation did not cause any increase in scorzonera root weight. According to Tekrony and Egli (1991), total emergence, rate of emergence, and the uniformity of emergence can potentially affect yield. Total emergence determines plant density, and there is a strong relationship between plant density and yield.

CONCLUSIONS

The results of the 2-year study presented in the paper demonstrated that irradiation of scorzonera seeds with He-Ne laser had a positive effect on the germination capacity, emergence and, partly, on root yield relative to the control. The most beneficial effects were noted after the 5-time irradiation of the seeds. Depending on the radiation dose applied, the germination capacity of the seeds increased by 1.5-13.2%. Scorzonera seedlings obtained from irradiated seeds had longer radicle and greater dry weight compared to those that grew from non-irradiated seeds. In the experiment the weather conditions proved to be a highly significant factor modifying the emergence and the yield of roots. The favourable weather conditions in 2013, with suitable sums of precipitations, caused an increase of those parameters in comparison with 2012. The root yield increased by 1.2-13.5% depending on the year of the studies and the laser light dose applied. The increase of root yield was related with the increased field emergence. The weight of scorzonera roots did not increase under the effect of laser stimulation of seeds. The obtained results show that laser irradiation can be used for the improvement of the quality of scorzonera seeds. Due to the fact that this method is safe for the environment, it can be applied in organic farming.

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WPŁYW NAŚWIETLANIA LASEROWEGO NA KIEŁKOWANIE NASION I PLON KORZENI SKORZONERY (*SCORZONERA HISPANICA* L.)

Marcela Krawiec[1], *Agata Dziwulska-Hunek*[2], *Salwina Palonka*[1], *Magdalena Kapłan*[1], *Piotr Baryła*[1],

[1]Katedra Nasiennictwa i Szkółkarstwa Ogrodniczego, Uniwersytet Przyrodniczy w Lublinie
ul. Leszczyńskiego 58, 20-680 Lublin
[2]Katedra Fizyki, Uniwersytet Przyrodniczy w Lublinie, ul. Akademicka 13, 20-950 Lublin
e-mail: marcela.krawiec@up.lublin.pl

Streszczenie: W uprawie skorzonery często występującym problemem są słabe, powolne i niewyrównane wschody, które występują pomimo użycia do siewu nasion o wysokiej zdolności kiełkowania. Celem badań było określenie wpływu przedsiewnej stymulacji laserowej nasion na kiełkowanie, wschody oraz plonowanie skorzonery. Nasiona naświetlano laserem He-Ne o długości fali 632,4 nm. Zastosowano dwie dawki promieniowania wiązką w zależności od gęstości powierzchniowej mocy: 6, i 8 $mW \cdot cm^{-2}$. Nasiona naświetlane były 1-, 3-, i 5-krotnie, zaś czas każdorazowego naświetlania wynosił ok. 0,1 s. Traktowanie nasion światłem lasera wpłynęło na zwiększenie zdolności kiełkowania, wzrost długości korzenia i suchej masy siewki, poprawę wschodów polowych i częściowo zwiększenie plonu ogólnego korzeni. W zależności od zastosowanej dawki napromieniowania zdolność kiełkowania nasion zwiększyła się o 1,5-13,2% w stosunku do kontroli. Najbardziej korzystny wpływ na wzrost zdolności kiełkowania i liczebności wschodów miało 5-krotne naświetlanie laserem. Zwiększenie plonu ogólnego korzeni w następstwie przedsiewnego napromieniowania nasion było związane ze zwiększeniem liczebności wschodów.

Słowa kluczowe: wschody, zdolność kiełkowania, światło lasera He-Ne, siewki