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Original Research

Effect of Electromagnetic Stimulation on Selected *Fabaceae* Plants

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Abstract

The effects of short exposure of seeds of lupine (*Lupinus* L.) and lucerne (*Medicago sativa* L.) to a low-frequency magnetic field (f=50 Hz, B= 30 mT), laser-light radiation (λ = 632.8 nm), and their combination on germination parameters and the content of photosynthetic pigments were examined. Independent studies were done on Petri dishes and in pots. As shown by data, short-term pre-sowing treatment with the physical factors mentioned above in most cases neither influences the germination parameters of the examined plants (no statistical differences between most of the samples) nor produces any negative effects.

Generally, an increase in the content of photosynthetic pigments was observed for lupine while no statistically relevant effect of pre-sowing stimulation on lucerne was observed in the laboratory experiment. The pot experiment on lucerne showed statistically relevant differences.

Comparing our data and the results obtained by other groups, we conclude that a short-term stimulation of selected *Fabaceae* with electromagnetic factors does not significantly influence the germination parameters or the content of their photosynthetic pigments. No statistical differences were found between coarse-grained and small-seed plants.

Keywords: alternating magnetic field, germination, laser light, lucerne, lupine, photosynthetic pigments

Introduction

The *Fabaceae* (leguminous plants), commonly known as the bean or pea family, is a large and economically important family of flowering plants [1]. Plants from this family are found throughout the world, growing in many different environments and climates and are important agricultural plants that play a major role in crop rotation through their positive effects on soil fertility, soil structure, and reduced soil erosion. They are an effective source of nitrogen fixation, able to produce high yields without nitrogen fertilization, and energy-effective crops to grow. They are major components of pastures and are harvested as hay for animal feeds.

In our research two plants from this family were selected, representing small-seed and coarse-grained plants such as lucerne (alfalfa) and lupine, respectively.

Alfalfa is one of the most important forage crops in the World. It has the highest yield potential and one of the highest feeding values of all adapted perennial forage legumes [2]. Its high level of digestible protein makes it an extremely valuable feed. It is a versatile crop that can be used as pasture, hay, silage, green crop, and as a cash crop. From a nutritional standpoint, it is an ideal complement to corn

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silage, which is relatively low in protein. When good quality alfalfa is included in the feed ration, it can reduce or eliminate the need for protein supplements. In addition, its relatively high levels of calcium, phosphorus, and magnesium are important in balancing mineral requirements of livestock.

Lupines are cultivated as forage and grain legumes. Lupine seeds have the full range of essential amino acids and they, contrary to soy, can be grown in more temperate to cool climates. They are becoming increasingly recognized as a cash crop alternative to soy [3].

The most frequent concern of producers is obtaining the highest possible yield per hectare. Of importance is the process of seed germination and the early stages of its growth.

The modern agricultural technologies involve intensive search for means leading to the improvement of the seed germination process, which influences plant development, growth, and the final crop.

An increase of interest in the influence of the pre-sowing effect of electromagnetic radiation on seed germination has been observed in recent years. New methods of refining of the sowing material are therefore welcome. Physical factors such as electromagnetic radiation are among the most popular in recent years (magnetic field, laser radiation, gamma radiation, and more), mainly because these methods are considered environmentally safer than chemicals (chemical dressings, growth regulators, etc.), which is of great importance in the case of so-called ecological agriculture [4-6]. Plant productivity depends on photosynthesis, which modifies the synthesis of organic substances [7]. Therefore, the level of photosynthetic pigments can be an indication of changes in plant physiology.

The aim of the study presented in this paper was to determine the effect of short exposure to low frequency electromagnetic fields (produced by laser light or alternating magnetic field) on germination parameters and photosynthetic pigments in selected *Fabaceae* plants representing small-seed and coarse-grained species such as lucerne (*Medicago sativa* L.) and lupine (*Lupinus luteus* L. and *Lupinus angustifolius* L.), respectively. The innovative element in the presented study was the application of a combination of laser light and magnetic field treatments for pre-sowing stimulation of lucerne and lupine seeds. Germination parameters as well as the pigment content were studied in two independent experimental sets conducted either in Petri dishes or in pots.

Material and Method

Experimental material consisted of the two kinds of lupine seeds (yellow lupine – *Lupinus luteus* L. and narrow-leaf sweet lupine – *Lupinus angustifolius* L. cv Baron) and two kinds of lucerne seeds (*Medicago sativa* L. cultivars Sitel and Legend) collected in earlier years. Lucerne seeds were three years older than lupine seeds. The relevant seeds of lupine and of lucerne were divided into four groups (100 seeds each) and kept in darkness for 24 hours. First, an untreated group was taken as a control sample (C). The second group was subjected to He-Ne laser light radiation (λ = 632.8 nm), the third to radiation with or 30 mT and 50 Hz alternating magnetic field (F), finally the fourth with the combination of laser light and magnetic field (L+F). In the case of the Petri dish experiment (laboratory experiment), the three-fold He-Ne laser light radiation of surface power density of 3 $mW \cdot cm^{-2}$ during the seed free fall was used and 15 s of radiation with 30 mT and 50 Hz alternating magnetic field. In the independent pot experiment the two-fold He-Ne laser light radiation of surface power density of 10 $mW \cdot cm^{-2}$ during the seed free fall was used along with 30 s of radiation with 30 mT and 50 Hz alternating magnetic field.

In the experiments the following experimental stands were used: laser irradiation stand constructed by Koper and Dygdała [8], and magnetic field stimulating stand constructed by Pietruszewski [9].

The laboratory experiment was carried out in air-conditioned laboratory premises at 20ºC±1ºC. Seeds were sown on Petri dishes lined with blotting paper moistened with distilled water.

The pot experiment was conducted in pots filled with potting for sowing. Pots were placed in air-conditioned laboratory premises at 20ºC±1ºC. 30 seeds per pot were used. Pots were watered with distilled water. In both cases we used automatic light control set to a cycle of 12/12 hours.

In the case of the laboratory and pot experiments, germination energy was determined according to the standard PN-R-65950:1994 after 5 or 4 days for lupine and lucerne, respectively.

Chlorophyll and carotenoid content was determined after 14 days of plant cultivation. Pigments were extracted from plant cotyledons in darkness with 80% acetone solution. 0.01% (v: v) of butylated hydroxytoluene (BHT) was added to avoid sample oxidation. Samples were vigorously shaken in the darkness for 15 minutes, filtered, and subjected to spectroscopic measurements. UV-Vis spectra of pigment isolates were performed using a double-beam Varian Carry Bio 300 spectrophotometer. Photosynthetic pigments were determined according to the procedure of Lichtenthaler and Buschmann [10]. Each measurement was done in four repetitions.

The Fischer (NIR) statistical test with the significance level of α = 0.05 was applied for the processing of the obtained experimental data.

Results and Discussion

Tables 1 and 2 show the germination parameters of lupine and lucerne seeds determined under laboratory conditions as well as for plants grown in pots. As shown by the data obtained, in most cases the short-term pre-sowing treatment with the abovementioned physical factors either does not influence the germination parameters of the examined plants (no statistical differences between most of the samples) or produces negative effects.

Table 1. Parameters of germination of lupine seeds cultivated on Petri glass (laboratory test) or in pots (pot test).

Lupine				
Laboratory test (Petri dishes)				
Physical factors	Yellow		Angustifolius	
	Germination energy (after 5 days) [%]	Germination capacity (after 10 days) [%]	Germination energy (after 5 days) [%]	Germination capacity (after 10 days) [%]
C	67.50[a]±2.08	82.50[a]±2.38	22.00[a]±2.58	40.00[a] ±4.16
L	73.75±1.50	90.50[b]±3.42	26.50[b]±2.38	48.25[b]±5.32
F	77.00[c]±5.48	84.00±2.58	31.25±4.65	46.25±2.63
L+F	70.50±4.20	85.50±1.29	26.75±5.91	46.00±4.83
Pot test				
C	82.50[a]±1.15	72.50±1.55	65.00±1.81	50.00[a]±2.65
L	75.00±1.47	65.00±1.85	57.50±2.17	27.50[b]±4.09
F	82.50±1.36	60.00±2.08	55.00±2.27	37.50±3.27
L+F	60.00[d]±2.08	57.50±2.17	62.50±2.36	52.50±2.62

C – control, untreated seeds, L – seeds subjected to laser stimulation, F – seeds stimulated with alternating magnetic field, L+F – seeds subjected to both laser and magnetic field stimulation.
Means in the same columns marked with different letters are significantly different, $P \leq 0.05$

Table 2. Parameters of germination of lucerne seeds.

Sowing Lucerne				
Laboratory test				
Physical factors	cv Sitel		cv Legend	
	Germination energy (after 4 days) [%]	Germination capacity (after 10 days) [%]	Germination energy (after 4 days) [%]	Germination capacity (after 10 days) [%]
C	65.50±3.65	71.50±1.00	48.50±2.38	54.25±2.22
L	62.25±7.37	66.00±6.16	40.25 ±6.29	51.25±3.40
F	63.00±5.83	67.00±4.40	42.00±2.45	49.50±4.93
L+F	61.00±9.09	66.25±9.11	45.50±9.29	50.50±9.57
Pot test				
C	53.33[a]±15.28	66.66[a]±8.82	13.33[a]±11.55	20.00±17.64
L	31.11±10.71	41.11[b]±7.70	23.33±5.77	26.67±8.82
F	23.33[b]±3.34	32.22[c]±9.62	21.11±1.92	32.22±5.09
L+F	27.78[c]±15.75	44.45[d]±15.75	31.11[d]±8.39	33.33±5.77

Explanations as in Table 1.

In the accessible literature sources there are not many experiments listed on plants belonging to the *Fabaceae* family. Experiments previously done on small-seed species primed with laser light of similar parameters such as lucerne cv Radius [11, 12], cv Legend [13], or on white clover showed no or very poor effect [14]. The only accessible data regarding field experiments carried out on mature lucerne plant comes from Ćwintal et al. [15], where the authors state no statistically significant effects of laser priming on dry matter, crude protein, crude fibre, or on the content of P, K, Ca, and Mg.

Measurements on germination of white lupine in pots were done previously by Podleśny and Podleśna [16]. Those authors registered a two-fold increase of the laser light pre-treated lupine plant emergence after 10 days (equivalent to germination capacity) under similar temperature conditions. The difference between our experiments was that they applied NPK fertilization to each pot filled

Table 3. Analysis of photosynthetic pigments content in lupine treated with pre-sowing electromagnetic stimulation methods.

Lupine						
Laboratory test						
Physical factors	Yellow			Angustifolius		
	Chlorophyll *a* [$\mu g \cdot g^{-1}$]	Chlorophyll *b* [$\mu g \cdot g^{-1}$]	Carotenoids [$\mu g \cdot g^{-1}$]	Chlorophyll *a* [$\mu g \cdot g^{-1}$]	Chlorophyll *b* [$\mu g \cdot g^{-1}$]	Carotenoids [$\mu g \cdot g^{-1}$]
C	247.34±1.66[a]	77.11±0.92[a]	93.25±1.11[a]	325.59±0.57[a]	107.86±2.48[a]	98.87±1.69[a]
L	339.91±1.75[b]	103.14±1.07[b]	106.66±0.98[b]	383.77±6.27[b]	143.92±2.20[b]	106.50±2.04[b]
F	373.56±4.60[c]	124.22±8.79[c]	111.90±2.72[c]	564.68±4.42[c]	185.77±9.24[c]	172.10±0.86[c]
L+F	321.36±1.82[d]	97.81±1.15[bd]	102.47±0.66[d]	395.10±8.01[d]	125.44±5.87[bd]	125.24±1.21[d]
Pot test						
C	565.92±105.10[a]	204.90±37.11[a]	101.25±14.03[a]	589.37±137.64	220.99±45.37	173.47±53.66
L	811.90±32.14[b]	264.93±33.04[b]	165.15±13.31[b]	591.34±76.73	214.76±31.41	167.87±17.33
F	599.11±43.79	237.37±21.52	115.39±6.07	714.59±303.87	242.96±88.42	204.40±95.68
L+F	438.24±90.92	165.40±27.50	67.62±22.63[d]	687.65±60.60	244.94±17.02	188.32±12.16

Explanations as in Table 1.

Table 4. Analysis of photosynthetic pigments content in lucerne treated with pre-sowing electromagnetic stimulation methods.

Sowing Lucerne						
Laboratory test						
Physical factors	cv Sitel			cv Legend		
	Chlorophyll *a* [$\mu g \cdot g^{-1}$]	Chlorophyll *b* [$\mu g \cdot g^{-1}$]	Carotenoids [$\mu g \cdot g^{-1}$]	Chlorophyll *a* [$\mu g \cdot g^{-1}$]	Chlorophyll *b* [$\mu g \cdot g^{-1}$]	Carotenoids [$\mu g \cdot g^{-1}$]
C	423.21±27.00	140.85±13.64	96.14±6.09	414.64±75.87	169.32±27.08	59.23±10.79
L	408.38±36.72	137.44±4.32	91.91±11.90	435.16±28.57	173.98±14.54	70.05±11.02
F	448.22±12.60	151.57±5.10	98.64±6.76	468.25±29.06	186.24±5.94	69.40±7.59
L+F	421.59±14.56	139.58±6.86	95.35±1.71	479.23±28.30	184.08±14.56	71.16±3.37
Pot test						
C	563.96±68.95	203.72±25.02	116.81±21.42	540.99[ac]±25.90	188.28[a]±12.56	125.88[ab]±5.45
L	533.03±84.38	188.10±29.40	115.20±19.52	578.79[b]±10.72	203.56[ab]±1.10	126.56[b]±9.01
F	604.90±88.40	215.32±31.55	129.63±18.20	390.80[c]±12.63	136.15[c]±1.99	85.56[c]±4.67
L+F	574.00±69.70	201.64±20.42	123.59±16.20	505.92±136.80	184.07[ad]±41.31	104.50±38.17

Explanations as in Table 1.

with a mixture of garden soil and sand, while we used no fertilizers and pots filled with potting for sowing. No light conditions were given.

Experiments carried out on lentils (*Lens culinaris*) treated with stationary magnetic fields [17] showed no significant differences concerning germination parameters. In another experiment, 1800 MHz electromagnetic waves emitted by cell phones applied on lentil seeds affected them only in the state of dormancy but not during germination [18]. Experiments done on soybeans after pre-sowing seed treatment with stationary magnetic fields of 2.9-4.6 mT and times of exposure up to 30s showed an increase of activity of superoxide dismutase (SOD) in soybean roots [19].

A slight increase in the germination rate of pea (*Pisum sativum* L.) cv. Kiler after laser priming of the power density of 6 and 8 mW/cm^2 was demonstrated [20]. Treatment of pea with stationary magnetic field over 7 and 14 days resulted in an increase of its growth parameters [21]. Alternating magnetic field stimulation (50 Hz and 60, 120, or 180 mT) resulted in an increased growth of pea seedlings [18]. Measurements of all groups were carried out under similar laboratory conditions.

Laser light pre-sowing treatment of faba bean produced an increase of its rate of growth [22] and affected the activity of amylolytic enzymes in the seeds, especially in the germination period [7].

Long-term exposure of *Phaseolus vulgaris* L. to static magnetic field (5, 10, 30, and 60 mT and exposure time up to 6 hours) showed an increase of its germination rate [23]. Interestingly, none of the authors reported treatment of the seeds with the combination of laser light and magnetic field.

Tables 3 and 4 show the effect of pre-sowing treatment with laser light or/and magnetic field on the content of photosynthetic pigments as compared to control, untreated sample. The content of photosynthetic pigments can be an indication of physiological productivity of the examined plants.

Generally, an increase of the photosynthetic pigments content was observed for lupine (the tendency to decrease was observed only in the case of pot test and stimulation with both physical factors; no statistical difference as compared to control). In the case of the laboratory experiment on lupine the increase in the content of photosynthetic pigments was correlated in most cases with the increase in germination parameters. Contrary to lupine there was no statistically significant effect of pre-sowing stimulation on lucerne during the laboratory experiment, while the pot experiment showed statistically relevant differences. No correlation was found between the level of photosynthetic pigments and the germination parameters for the pot experiment. Such effects are difficult to explain, as usually the higher pigment content in seeds stimulated with laser light should result from increased efficiency of photosynthesis [24] indicated by higher germination parameters. The results obtained for lucerne can be explained in terms of the effect of cultivar, i.e. the genetic factor. Lucerne cv. Sitel differs morphologically from cv Legend. The first one has 3 leaves per sprout while the second one can have as many as 7 leaves per sprout.

No data exist on the content of chlorophyll and carotenoids for the species examined in the presented study. The reports concerning pea subjected to laser priming or alternating magnetic field show insignificant changes in the chlorophyll content in spite of a statistically relevant increase in the growth rate of pea seedlings [18, 20].

Conclusions

The effect of short-term pre-sowing treatment with physical factors such as stimulation with laser light, magnetic field, or both factors in most cases does not influence the germination parameters of the small-seed lucerne or coarse-grained lupine plants (no statistical differences between most of the samples). Generally, an increase of the photosynthetic pigments content was observed for lupine, while no statistically relevant effect of pre-sowing stimulation on lucerne was observed in the laboratory experiment. The pot experiment on lucerne showed statistically relevant differences.

Comparing our data and the results obtained by other groups, we conclude that a short-term stimulation of selected *Fabaceae* with electromagnetic factors does not significantly influence the germination parameters or the content of photosynthetic pigments. No differences were observed between coarse-grained and small-seed plants.

References

1. LEWIS G., SCHRIRE B., MAC KINDER B. and LOCK M. (eds). Legumes of the world. Royal Botanical Gardens, Kew, UK. **2005**.
2. TYROLOWÁ Y., VÝBORNÁ A. Effect of the stage of maturity on the leaf percentage of lucerne and the effect of additives on silage characteristics. Czech J. Anim. Sci. **53**, (8), 330, **2008**.
3. DIJKSTRA D.S., LINNEMANN A.R., Van BOEKEL T.A.J.S. Towards sustainable production of protein-rich foods: Appraisal of eight crops for Western Europe. Part II. Analysis of the technological aspects of the production chain. Critical Reviews in Food Sci. Nutr. **43**, 481, **2003**.
4. PIETRUSZEWSKI S., MUSZYŃSKI S., DZIWULSKA A. Electromagnetic fields and electromagnetic radiation as non-invasive external stimulants for seeds (selected methods and responses). Int. Agrophysics **21**, 95, **2007**.
5. HERNANDEZ A.C., DOMONGUEZ P.A., CRUZ O.A., IVANOV R., CABALLO C.A., ZEPEDA B.R. Laser in agriculture. Int. Agrophysics **24**, 407, **2010**.
6. ROCHALSKA M., GRABOWSKA-TOPCZEWSKA K, MACKIEWICZ A. Influence of alternating low frequency magnetic field on improvement of seed quality. Int. Agrophysics, **25**, 265, **2011**.
7. PODLEŚNY J. Effect of laser irradiation on the biochemical changes in seeds and the accumulation of dry matter in faba bean. Int. Agrophysics **6**, 209, **2002**.
8. KOPER R., DYGAŁA Z. Pre-sowing laser treatment stand for seeds. Patent UPRP, No. 162598, WUP No. **12**, 1111, **1993** [In Polish].
9. PIETRUSZEWSKI S. Electromagnet. Utility model, UPRP, Protection right No. 59863, WUP No. **7**, 1077, **2003** [In Polish].
10. LICHTENTHALER H.K., BUSCHMANN C. Chlorophylls and Carotenoids: Measurement and Characterization by UV-Vis Spectroscopy. In: Current Protocols in Food Analytical Chemistry, Wiley & Sons, Inc., USA., Supplement **1**, F4.31-F4.3.8, **2001**.
11. DZIWULSKA A. Effect of pre-sowing laser stimulation on sowing value of lucerne seeds. Acta Sci. Pol., Technica Agraria **5**, (3), 37, **2006** [In Polish].
12. DZIWULSKA A., KOPER R. Effect of pre-sowing laser biostimulation on germination of lucerne seeds. Acta Agrophysica **82**, 33, **2003** [In Polish].
13. WILCZEK M. KOPER R., ĆWINTAL M., KORNIŁŁOWICZ-KOWALSKA T. Germination capacity and health status of alfalfa seeds after laser treatment. Int. Agrophysics **19**, 85, **2005**.
14. DZIWULSKA A., KOPER R., WILCZEK M. Estimation of effect of He-Ne laser beam on germination capacity of white clover variety of Anda cultivar. Acta Agrophysica **3**, (3), 435, **2004** [In Polish].
15. ĆWINTAL M., DZIWULSKA-HUNEK A., WILCZEK M. Laser stimulation effect of seeds on quality of alfalfa. Int. Agrophysics **24**, 15, **2010**.

16. PODLEŚNY J., PODLEŚNA A. Morphological changes and yield of selected species of leguminous plants under the influence of seed treatment with laser light. Int. Agrophysics **18**, 253, **2004**.
17. ALADJADJIAN A. Influence of stationary magnetic field on lentil seeds. Int. Agrophysics **24**, 321, **2010**.
18. IQBAL M., HAQ Z.U., JAMIL Y., AHMAD M.R. Effect of presowing magnetic treatment on properties of pea. Int. Agrophysics **26**, 25, **2012**.
19. ÇELIK Ö., BÜYÜKUSULU N., ATAK Ç., RZAKOULIEVA A. Effect of Magnetic Field on Activity of Superoxide Dismutase (SOD) and Catalase in *Glycine max* (L.) Merr. Roots. Pol. J. Environ. Stud., **18**, 175, **2009**.
20. KRAWIEC M., DZIWULSKA-HUNEK A. Effect of pre-sowing laser stimulation on germination of pea seeds (*Pisum sativum* L.) and field emergence. Zeszyty Postępowe Problemów Nauk Rolniczych **539**, 359, **2009** [In Polish].
21. MARTINEZ E, FLÓREZ M., MAQUEDA R., CARBONELL M.V., AMAYA J.M. Pea (*Pisum sativim*, L.) and Lentil (*Lens culinaris*, Medik) Growth Stimulation Due to Exposure to 125 and 250 mT Stationary Fields. Pol. J. Environ. Stud., **18**, (4), 657, **2009**.
22. PODLEŚNY J., MISIAK L., KOPER R. Concentration of free radicals in faba bean seeds after pre-sowing treatment of the seeds with laser light. Int. Agrophysics **15**, 185, **2001**.
23. ODHIAMBO J.O., NDRITU F.G., WAGARA I.N. Effects of Static Electromagnetic Fields at 24 hours Incubation on the Germination of Rose Coco Beans (*Phaseolus vulgaris* L.). Romanian J Biophys. **19**, (2), 135, **2009**.
24. PASTORE D. MARTINO C., BOSCO G., PASSARELA S. Stimulation of ATP synthesis via oxidative phosphorylation in wheat mitochondria irradiated with helium-neon laser. Biochem. Molecular Biol. Int. **39**, (1), 149, **1996**.