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Original Research

Short-Term Exposure to Pre-Sowing Electromagnetic Radiation of Amaranth Seeds Affects Germination Energy but not Photosynthetic Pigment Content

Agata Dziwulska-Hunek, Agnieszka Sujak*, Krzysztof Kornarzyński

Department of Physics, University of Life science in Lublin, Akademicka 13, 20-950 Lublin, Poland

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Abstract

The effects of short-term (30 s) exposure of amaranth seeds to low frequency magnetic field (f=50 Hz, B=30 mT), laser light radiation ($\lambda = 632.8$ nm), or to their combination on germination, plant height, and the content of photosynthetic pigments were examined. The conducted experiment consisted of three independent parts:

1 – laboratory experiment where seed germination was carried out on Petri dishes
2 – pot experiment
3 – field experiment

Pre-sowing radiation of the amaranth seeds with a single physical factor resulted in the statistically significant increase of the germination energy as compared to control. Radiation of the seeds with the combination of laser light and magnetic field induced opposite response in the case of laboratory and pot tests: significant increase of the germination energy was observed for laboratory tests while a decrease of this parameter was observed for the pot test. Pre-sowing radiation treatments did not influence germination capacity, which indicated that only early stages of the germination process were affected. Although no statistical differences were found, the results indicate that pre-sowing radiation of the seeds with a single factor resulted in an increase in the number of plants per 1m^2 in field experiment.

Contents of carotenoids and chlorophylls were not affected by electromagnetic radiation. The experiments indicated a stable chlorophyll *a* to chlorophyll *b* ratio of c.a. 3.5.

An attempt to explain on the molecular level the influence of electromagnetic radiation on germination and content of photosynthetic pigments in amaranth was made.

Keywords: alternating magnetic field, amaranth seeds, germination, laser light, photosynthetic pigments

Introduction

In recent years the increase of interest in the influence of the pre-sowing effect of electromagnetic radiation on seed germination has been observed concerning intensive search for modern agricultural technologies [1]. The aim of the plant food producers is the improvement of the seed germination process, which influences plant development, growth, and the final crop. Therefore, the non-destructive methods of refining the sowing material are welcome. Physical factors have become popular in recent years 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-

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*e-mail: agnieszka.sujak@up.lublin.pl

called ecological agriculture [2, 3]. The most popular techniques are those based on usage of electromagnetic radiation of different wavelengths, frequencies, and exposure times. The various groups use static [4-5] or alternating low [6-10] or high [11] frequency magnetic fields of different strengths and doses.

Researchers also use light of different wavelengths ([12] for the reviews see [2, 13]), γ-radiation [14], cold plasma and radio-wave pre-treatments [15-17]. The combinations of some of these factors are also being verified [18-20].

As indicated by the previous experiments, amaranth seeds germinate in field conditions after 4-5 days under an optimal temperature of 8-10ºC. Plant emergence can take as long as 12-14 days when subjected to low temperatures and low soil humidity; intensive plant growth occurs after 3-4 weeks [21]. The experiments with germination of amaranth cv Rawa under laboratory conditions were also made. The results showed that the optimal germination temperature for this cultivar is within 20-35ºC and that the maximum germination rate is observed for 35ºC [22].

Seeds of the amaranth plant are characterized with great nutritional qualities such as high protein content (16-18%) and nutritionally welcomed amino acid composition. Amaranth protein is rich in lysine, tryptophan, and methionin in contrast to other cereals [23, 24]. It also contains valuable minerals and amino acid composition. Interestingly, application of the non-invasive pre-sowing laser or magnetic field stimulation can improve its nutritional values [19, 25]. As shown by our previous experiments, application of the pre-sowing prompting with the electromagnetic fields resulted in the significant increase in the levels of zinc and iron, and also in an increase of essential fatty acids [25]. An increase in certain amino acids was also observed [19]. The exact mechanisms of such phenomena are not yet explained.

We hypothesize that the biological processes occurring after laser or magnetic light pre-sowing prompting of the seeds can be connected with the development of a photosynthetic apparatus that can be monitored by a change in composition of the specific photosynthetic pigments. Therefore, we decided to determine the influence of electromagnetic radiation on the amaranth seed germination indices (such as germination energy and germination capacity after three and 14 days, respectively) and on the content of photosynthetic pigments in seedlings after 14 days of cultivation under laboratory (Petri dish experiments and pot experiments) and field conditions.

Materials and Methods

Experimental material consisted of the amaranth seeds cv Rawa divided into four groups (100 seeds each). Seeds were kept in darkness for 24 hours. The first untreated group was taken as a control sample (C). The second group was subjected to three-fold He-Ne laser light radiation ($\lambda = 632,8$ nm) of surface power density 6 mW·cm^{-2} (L) during the seed free fall, third to 30s of radiation with 30 mT and 50 Hz alternating magnetic field (F), and finally the fourth with a combination of laser and magnetic fields (L+F).

In the experiments the following experimental stands were used: a laser irradiation stand constructed by Koper and Dygdała [26], and a magnetic field stimulating stand constructed by Pietruszewski [27].

The conducted experiment consisted of three independent parts:

1. laboratory experiment where seed germination was carried out on Petri dishes
2. pot experiment
3. field experiment

A laboratory experiment was carried out in air conditioned laboratory premises at 20±1ºC. Amaranth 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 20±1ºC. Thirty amaranth seeds per pot were used. Pots were watered with distilled water.

Field experiments were performed in Felin (near Lublin, Poland) with the application of random squares methods and were conducted in four repetitions.

Experiments were conducted on light silty soil originated from loess soil characterized by a high amount of fine silt, which keeps the water very well, is not susceptible to heating, and has a tendency to form a crust after the rainfall. Prior sowing of the soil was fertilized with 46% of urea nitrate. Forty seeds were sown per 1 m^2. Distance between seeds in rows was 5 cm and the distance between separate rows was 40 cm. For sowing, a manual seeder was applied.

In the case of laboratory and pot experiments, germination energy (after 3 days) was determined according to the PN-R-65950:1994 standard. In field experiments germination capacity (after 14 days according to the same standard) was determined. For field experiments the growing pattern per 1 m^2 after 10 weeks of cultivation was evaluated in order to estimate the plant survival rate.

In the case of pot and field experiments, plant height was determined respectively after 4 and 10 weeks of cultivation. Height measurements were done on three randomly chosen plants in each row.

Chlorophyll and carotenoid content was determined exclusively for laboratory and pot experiments after 14 days of cultivation. Pigments were extracted from amaranth cotyledons in darkness with 80% acetone solution. Butylated hydroxytoloyene (BHT; 0.01%, v:v) was added to avoid sample oxidation. Samples were vigorously shaken in 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 [28], with slight modifications. Each measurement was done in four repetitions.

Fischer (NIR) Statistical Test with a significance level of $\alpha = 0.05$ was applied for processing the obtained experimental data.

Table 1. Parameters of germination (±S.D) of amaranth seeds under laboratory, pot, and field test conditions.

Specification		Physical factors			
		C	L	F	L+F
Laboratory test	Germination energy (after 3 days) [%]	12.67^{A}±0.58	22.33^{B}±5.69	23.33^{C}±11.24	17.67^{D}±6.03
	Germination capacity (after 14 days) [%]	67.33±6.81	67.33±1.53	69.00±3.61	63.00±1.73
Pot test	Germination energy (after 3 days) [%]	44.33^{A}±5.13	45.67^{B}±2.31	51.00^{C}±8.54	33.00^{D}±0.00
	Germination capacity (after 14 days) [%]	82.33±4.04	81.00±1.73	79.00±3.46	83.33±3.51
Field test	Germination capacity [%]	36.67±10.40	45.81±17.55	46.68±7.23	37.51±6.62
	Growing pattern [%]	41.68±13.77	60.83±23.78	53.32±10.10	41.67±2.87

C – control sample
L – sample subjected to 3-fold pre-sowing radiation with laser He-Ne divergent beam of 6 $\mathrm{mW\cdot cm^{-2}}$
F – sample subjected to magnetic field with magnetic flux density of 30 mT and time of exposure – 30s
L+P – pre-sowing treatment with laser light combined with magnetic field; as specified above
A→D – level of significance $\alpha = 0.05$

Results and Discussion

Table 1 compares the parameters of germination of amaranth seeds after pre-sowing treatment with physical factors such as laser light (L), alternating magnetic field (F), or a combination of these factors (L+F) as compared to control (C) for three independent experiments (laboratory test, pot test, and field experiment).

Untreated seeds were characterized by a different germination energy, equivalent to a number of seeds that germinated properly after 3 days under defined conditions. It was not possible to determine this parameter for the seeds under field test. Germination energy for the seeds grown in pots was much higher than this for laboratory test on Petri dishes under the same temperature and light conditions. Such a phenomenon can be explained in terms of watering the seeds with distilled water lacking the minerals necessary for germination in the early stages. All the substances necessary for germination had to be taken from the seeds themselves. Potting for sowing used in pot experiments probably supplied these microelements to the germinating seeds. Pre-sowing treatment of the seeds with a single method such as laser light treatment or alternating magnetic field treatment with defined parameters resulted in the statistically significant increase of the germination energy in all the samples as compared to control. The increase of the germination rate for buckwheat [20], *Salvia officinalis* L. or *Calendula officinalis* L. [4] treated with a magnetic field was previously reported. The results obtained for wheat by different groups are contradictory [5, 29]. An increase in germination energy after laser treatment with the red light can be explained in different ways: as the simple supply of energy to the seed or in terms of plant physiology as the complex process of the excitation of the phytochrome or affecting the activity of amylolitic enzymes. In comparison with conventional light, laser light radiates in a coordinated manner (coherent) and is emitted almost in parallel. Live cells are able to absorb, transform, and use the energy of laser light photons.

Phytochrome is a photoreceptor, chemically a protein that plants use to detect light. It is sensitive to light in the red and far-red region of the visible spectrum. Many plants use it to regulate germination of seeds (photoblasty), elongation of seedlings, the size, shape and number of leaves, and the synthesis of chlorophylls. In principle, the processes determining the proper germination process are these leading to the generation of metabolic energy and to the formation of certain substrates for the further synthesis of other components in the cells of growing seedlings (imbibition, catabolic, and anabolic phases). However, they require certain amounts of water. The question arises whether the water available in dry amaranth seed (in our case as low as 9%) is sufficient to induce this processes or whether the laser treatment effect prolongs the phase of soaking seeds with water. It has been proved that pre-sowing irradiation of faba bean seeds with laser light results in a faster uptake of water and achieving the larger mass during seed imbibing in comparison to untreated seeds [30]. An increase in amylolitic enzymes was also reported in faba bean seeds after irradiation of the seeds with laser light of the same wavelength used in this study [31].

Increase in the germination energy in the case of stimulation of the seeds with alternating magnetic field may also present lots of difficulties. Experiments done on wheat indicate no effect of magnetic field on its germination [29]. At this stage it is difficult to explain this phenomenon on a molecular level, but one can assume again the process of energy supply or speculate about the ion cyclotron resonance effect where certain combinations of frequencies of magnetic and geomagnetic fields result in the excitation of frequencies characteristic for certain ions participating in the process of seed germination and early plant growth [31-33]. Numerous experiments were conducted regarding this effect on agricultural plants, especially on radish [6-8, 34] but also on barley [6], mustard [6], and bean [9]. Experimental layouts were mostly set to meet calcium resonance frequencies [9], but also potassium-tuned [34] and magnesium-tuned [8, 34] combined magnetic fields were

Table 2. Amaranth plant heights (±S.D.) obtained from pot or field experiment.

Specification		Physical factors			
		C	L	F	L+F
Pot test	Plant height* $[m] \cdot 10^{-2}$ per pot	10.83±1.04	11.30^{A}±0.98	10.97±1.46	4.13^{B}±7.16
Field test	Plant height** $[m] \cdot 10^{-2}$ per 1 m^2 plot	42.80±5.39	38.89±5.66	36.50±3.65	34.22±4.35

* measured after 4 weeks of cultivation
**measured after 10 weeks of cultivation
Other: as in Table 1

Table 3. Content of photosynthetic pigments [μg/g of green mass±S.D.] in amaranth cotyledons measured after 14 days of cultivation.

Specification		Physical factors			
		C	L	F	L+F
Laboratory test	Chlorophyll *a*	288.266±21.860	289.924±33.622	259.949±52.807	314.848±46.940
	Chlorophyll *b*	86.624±9.796	86.264±9.303	77.003±14.474	92.727±12.986
	Carotenoids	103.386±17.643	104.879±15.634	97.270±6.079	97.135±6.343
Pot test	Chlorophyll *a*	639.091 ±105.934	547.228±20.344	578.882±31.213	595.625±6.582
	Chlorophyll *b*	171.363±31.951	144.012±5.422	154.682±8.528	159.158±0.858
	Carotenoids	95.098 ±52.094	117.558±2.520	122.758±5.786	131.179±5.499

Abbreviations as in Table 1

applied. Much of this research has reported positive effects on germination or seedling growth, while a comparable number have reported negative or no effects.

Stimulation of the seeds with the combination of both physical factors caused an opposite response in the case of the laboratory test and pot test.

The statistically significant increase of the germination energy was observed in the case of a laboratory test while a statistically significant decrease of this parameter was observed for the pot test. Maybe stimulation with the combination of methods produces negative coupling of the phytochrome effect after the laser treatment and ion cyclotron resonance after magnetic treatment in the case when external sources of ions dissolved in water are available. In laboratory tests when no additional ions are available, the effect of response to the red light dominates. This problem therefore has to be studied separately with the carefully selected ions and defined field frequencies. The similar laboratory test was done on wheat and oat seed stimulated with cold plasma. No effect on the early stages of germination was observed for exposure times between 180 and 1,200 s, while a slight but not significant increase in the number of germinated seeds after 4 days was observed for the samples subjected to cold plasma pre-treatment for 2,400 s [15].

For all the tests the examined seed germination capacity was estimated. As shown by the test results, the highest germination capacity was found for pot test, followed by a laboratory test and field test. Similarly to germination energy, this parameter also can be explained in terms of growing conditions. Much lower germination capacity in the field experiment can result in a lower germination temperature as compared to laboratory and pot experiments, c.a. 10ºC and 20ºC, respectively. As shown by the previous experiments the optimal germination temperature for amaranth seeds cv Rawa on Petri dishes was 20ºC [7]. No statistically significant effects of electromagnetic stimulation on germination capacity of amaranth seeds were found. This clearly indicates that pre-sowing electromagnetic treatment of the parameters used in this study affects only the initial stages of the seed germination that additionally supports the explanation, including excitation of phytochrome. The effect of increase of the germination capacity was reported for wheat seeds by Rochalska [29]. Filatova et al. [16] showed the enhancement of laboratory and field germination of seeds of blue lupine, catgut, honey clover, and soy pre-treated with radio frequency plasma and also exposed to a high frequency electromagnetic field.

In the case of agriculture the most critical factor is the level of plant survival until full maturity stage, which is expressed in the growing pattern. Although no statistical difference was found, the results indicate that stimulation of the seeds with a single electromagnetic factor resulted in an increase in the number of plants per m^2.

The negative effects of the combination of light and magnetic field were observed for both pot and field experiments in the case of plant height (Table 2). Statistical differences were found only for pot test. Plant height as a parameter evaluating the influence of electromagnetic fields can, however, be questionable. In the case of long-stemmed plants, decreasing plant height can be an advantage, preventing the mature plant from lodging and thus leading to an increase of the final crop. The effect of decreasing plant height in the laboratory experiment for *Triticum aestivum* L. was observed previously for seeds pre-treated with cold plasma [17]. In our case the experimental layout didn't allow us to measure this feature for the laboratory test.

Table 3 shows the content of photosynthetic pigments in amaranth cotyledons for laboratory test and pot test. Chlorophyll content is correlated with nitrogen content in plants and with the process of extracting nitrogen and other macro- and micro-elements important for plant metabolism, as well as the content of low-molecular enzymes. In our study no statistically significant difference between control and samples subjected to the pre-sowing treatments were observed. The increase of chlorophyll content in leaves upon magnetic pre-sowing stimulation was previously reported for sugar beets [30]. The plants, however, were much older than these in our study. The process of adaptation and stabilization of the level of photosynthetic pigments in wheat plants pre-treated with cold plasma was reported [17].

As the results indicate, the content of chlorophylls was higher in the case of the pot test compared to the laboratory test; c.a. 2.0 and 1.8 times, respectively for chl *a* and *b*. Such a result is the consequence of normal enzymatic processes leading to synthesis of chlorophylls under conditions where uptake of microelements from the water or soil is possible. The relative increase in the ratio of chlorophyll *a* to carotenoids was observed in the samples from laboratory test. All the plants examined were characterized by a stable chlorophyll *a/b* ratio of 3.5±0.3 and also by the stable carotenoid content.

Conclusions

1. Pre-sowing radiation of the amaranth seeds with single factor (laser light or magnetic field) results in a statistically significant increase of the germination energy as compared to control.
2. Stimulation of the seeds with the combination of laser light and magnetic field causes opposite response in the case of laboratory and pot tests: a significant increase of the germination energy was observed for laboratory test while a significant decrease of this parameter was observed for pot test.
3. Pre-sowing radiation treatments do not influence germination capacity, which indicates that only early stages of the germination process are affected.
4. Although no statistical difference was found, the results indicate that pre-sowing radiation of the seeds with a single factor resulted in an increase of the number of plants per 1 m^2.
5. Contents of carotenoids and chlorophylls were not affected by electromagnetic radiation.
6. The molecular mechanisms leading to a different response of the seeds on pre-sowing treatment with using electromagnetic radiation is still unclear.

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