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Minerals and fatty acids of amaranth seeds subjected to pre-sowing electromagnetical stimulation

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A b s t r a c t. It was hypothesized that the pre-sowing electromagnetic stimulating methods with laser light or/and magnetic field applied to amaranth seeds may influence minerals uptake from the soil as well as the fatty acids composition. Electromagnetical simulation of the seeds resulted in a significant decrease of the levels of potassium, calcium magnesium, sodium, copper and manganese ($P \leq 0.01$). The stimulated seeds contained comparable amount of phosphorus. Interestingly, stimulation with both electromagnetical methods resulted in significant increase in the level of zinc and over two-fold increase in iron content in the seeds as compared to control. The ratio of K to Na increases upon electromagnetical stimulation in spite of a decrease in the level of these elements. In all the seeds mono- and polyunsaturated acids dominated in all tested fats. The electromagnetical stimulation of amaranth seeds resulted in the increase of essential fatty acids and the decrease in most of the saturated fatty acids which is highly demandable from the point of healthy nutrition.

K e y w o r d s: amaranth seeds, pre-sowing stimulation, laser light, magnetic field, minerals, fatty acids

INTRODUCTION

Amaranth (*Amaranthus cruentus*) belongs to the oldest cultivated plants. Today, cultivated not only in the countries of South America, amaranth has awoke the interest of nutrition experts, who call it the cereal of the 21st century. It is considered as a huge chance to satisfy nutrition needs (NRC, 1984). The lack of detailed data on the mineral content and composition of the lipid fraction of amaranth seeds (subjected to magnetic field pre-sowing stimulation) forced the authors to undertake a study on that subject.

The previously conducted experiments showed that the pre-sowing laser light and magnetic field stimulation is producing changes in the morphological features of the seeds. The chemical compositions of amaranth isolates and amino-acid composition of the proteins as well as the nutritive values were estimated (Sujak *et al*., 2009). The highest crude fat content (63.9 ± 1.5 g kg^{-1}) was found in control seeds. Although the samples varied in the mean values of crude fat, no statistical differences have been found between samples except the statistical difference between the sample subjected to both electromagnetical stimulation methods and other samples ($P \leq 0.01$). In general, the crude fat in all the samples was similar to the previously reported (Gamel *et al*., 2006; Saunders and Becker, 1984) but higher than reported in NRC (1984). The significant growth in crude ash content has been registered (*ca*. 20%) in the samples treated previously with electromagnetical stimulating methods (Sujak *et al*., 2009). Even in the control sample the ash content was higher than reported previously for amaranth in NRC (1984).

It was hypothesized that the pre-sowing electromagnetic stimulating methods may influence the minerals uptake from the soil what can be shown by the significant change in the content of minerals in the seeds of plants grown from the seeds stimulated prior to sawing.

The aim of this study was to check the influence of electromagnetical pre-sowing stimulation of the amaranth seeds on their minerals content and composition of the oil fraction.

MATERIALS AND METHODS

The experimental material comprised amaranth (*Amaranthus cruentus*) seeds cultivar Rawa was divided into 4 groups and treated as follows: first group (untreated) remained as a control (C), second was subjected to laser simulation (L – in five series during the free fall of seeds from the charging chopper chute) with the He-Ne light of λ =632.8 nm and density power of 6 mW cm^{-2}, third

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group (F) was stimulated with the alternating magnetic field (50 Hz) of an intensity of 30 mT during the exposure time of 30 s and the last group (L+F) subjected to both laser and magnetic field stimulation. The seeds were then sown on the experimental plot of $1m^2$ each (3.7 g of seeds per each plot). The spacing between the rows was approx. 0.1 m. After the plants were ripen (after 140 days), they were collected, the seeds plucked out, cleaned and rendered free of dust, then placed in tightly closed PVC test tubes at room temperature and subjected to analysis.

In order to determine the contents of mineral components and trace elements, the material (ground to fine powder with laboratory KNIFETEC 1095 sample mill, Foss Tecator) was subjected to wet digestion in a mixture of HNO_3 and $HClO_4$ according to the AOAC 986.15 standard (AOAC, 2000a). The levels of Na, K, Ca, Mg, Zn, Cu, Fe, and Mn and were recorded using flame AAS technique applying a 'Solaar 939' spectrometer (Unicam). Phosphorous was determined spectrophotometrically by using the modified colorimetric method of (Allport, 1956). PN -ISO 6491:2000. The determination error was 5%.

Fatty acids (C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, C18:1 cis, C18:2, C18:3 , C20:0, C20:1, C20:2, C20:3, C20:4, C22:0, C22:1, C24:0, C24:1) as methyl esters after preliminary fat saponification and acid esterification according to AOAC-969.33 and 963.22 procedures (AOAC, 2000b, 2000c) were determined by GC using a Unicam 610 gas chromatograph equipped with a flame-ionization detector and a 60 m (0.25 mm i.d.) column coated with a 0.25 m film of HP-23. A temperature gradient was applied (160°C for 1 min, then incremented by 2.75 to 215°C min, 215°C for 2 min, then incremented by 40 to 230°C min, 230°C for 2 min). The injection port and detector temperatures were 270°C, split ratio 1:50. Hydrogen was used as carrier gas at a flow rate of 1.3 ml min^{-1}. An internal standard technique (heptadecanoic acid) was applied. All reagents used for the extractions and derivations were of analytical reagent grade. Calculations were based on previously published analyses of standard mixture and calculation of the individual correction coefficients (Kowalski, 2007).

The admissible error for determinations of minerals and fatty acids was 5%. One-way analysis of variance was carried out on the experimental results using groups as an independent variable. The significance of differences between means was compared by Duncan multiple range tests. All calculations were performed using an ANOVA package from STATISTICA.pl.6.0.

RESULTS AND DISCUSSION

Table 1 shows the content of several minerals of the amaranth seeds. The control seeds contained higher levels of potassium (K = 8.92±0.25 g kg^{-1}), calcium (Ca = 2.30±0.01 g kg^{-1}), magnesium (Mg = 4.27± 0.007 g kg^{-1}), zinc (Zn = 93.77± 0.29 mg kg^{-1}), copper (Cu = 10.97±0.02 mg kg^{-1}), and manganese (Mn = 54.50±0.14 mg kg^{-1}), but lover levels of phosphorus (P = 1.19±0.01 g kg^{-1}), sodium (Na = 76.40±0.11 mg kg^{-1}), and iron (Fe = 85.37±3.78 mg kg^{-1}) than reported previously for amaranth grown in a greenhouse (Gamel *et al*., 2006). This however can be explained in terms of different cultivar as well as of growing under different conditions and the quality of soil.

Table 1 shows that electromagnetical simulation of the seeds resulted in a significant decrease of the levels of potassium (between 5.42 and 6.47 g kg^{-1}), calcium (1.19-1.61 g kg^{-1}) magnesium (2.99-3.63 g kg^{-1}), sodium (25.07-40.06 g kg^{-1}), copper (7.20-8.74 g kg^{-1}) and manganese (30.77-41.93 g kg^{-1}) ($P \leq 0.01$). The stimulated seeds contained comparable amount of phosphorus (between 1.15 and 1.27 g kg^{-1}), stimulation with laser light did not affect the phosphorus content, stimulation with magnetic field produced a slight decrease of the level of phosphorus, while stimulation by using both

T a b l e 1. Mineral content of amaranth seeds

Mineral	C	L	F	L+F
K (g kg^{-1})	8.92± 0.25^{a}	5.42± 0.01^{b}	6.10± 0.06^{c}	6.47± 0.03^{d}
Ca (g kg^{-1})	2.30± 0.01^{a}	1.23± 0.001^{b}	1.61± 0.001^{c}	1.19± 0.005^{d}
Mg (g kg^{-1})	4.27± 0.01^{a}	2.99± 0.002^{b}	3.63± 0.01^{c}	3.19± 0.01^{d}
P (g kg^{-1})	1.19± 0.01^{ab}	1.22± 0.01^{Ab}	1.15± 0.01^{c}	1.27± 0.02^{bdB}
Na (mg kg^{-1})	76.40± 0.11^{a}	25.07± 0.07^{b}	39.73± 0.21^{c}	40.06± 0.50^{d}
Zn (mg kg^{-1})	93.77± 0.27^{a}	58.83± 0.16^{b}	85.87± 0.24^{c}	100.93± 0.30^{d}
Cu (mg kg^{-1})	10.97± 0.02^{a}	7.20± 0.11^{b}	8.30± 0.06^{c}	8.74± 0.17^{cd}
Fe (mg kg^{-1})	85.37± 3.78^{a}	88.97± 1.56^{a}	84.77± 0.73^{a}	180.87± 0.54^{b}
Mn (mg kg^{-1})	54.50± 0.14^{a}	40.3± 0.28^{b}	41.93± 0.29^{cb}	30.77± 0.38^{d}

C – control seeds, L – seeds subjected to laser simulation, F – seeds stimulated with the alternating magnetic field, L+F – seeds subjected both to laser and magnetic field stimulation. Means in the same column with different letters are significantly different: a-d – $P \leq 0.01$, A-B – $P \leq 0.05$.

methods resulted in a slight increase of this mineral. Stimulation with laser light and electromagnetical field results in decrease of minerals such as zinc, copper iron and manganese.

The stimulation with both electromagnetical methods resulted in significant increase in the level of zinc and two-fold increase in iron content in the seeds as compared to control seeds, while application of a single method resulted in a significant decrease in the level of zinc but did not cause a significant change in the content of iron. In the case of chickling vetch seeds an increase of Fe in seed yield have been reported upon pre-sowing stimulation with red light radiation accompanied with nitragine dressing (Truchliński *et al*., 2002). 100 g of amaranth seeds cover 1/3 of daily demand for calcium and potassium (White and Broadley, 2005). Amaranth is a low sodium product (below 10 mg per 100 g). Sodium levels decrease upon electromagnetical stimu- lation (Champagne and Lastor, 2009). The amount of bone- strengthening Ca in amaranth seeds (119-230 mg kg^{-1}) is higher than in milk products (100 mg kg^{-1}) or in leguminous plants (*ca* 150 mg kg^{-1}) (NRC, 1984). Potassium is opposed by sodium, and the two positive ions are jointly balanced by the negative ion, chloride (Sorof *et al*., 1997). The ratio of K to Na grows upon electromagnetical stimulation in spite of a decrease in the level of these elements (K/Na = 117 for control and 216, 153, and 164 for laser stimulated, magnetic field stimlated and the seeds stimulated with both electromagnetic methods). With regard to the amount of iron (7.5 mg in 100 g) it leaves behind almost all other plants together with famed spinach (Łoś-Kuczera and Piekarska, 1988; NRC-NAS, 1989). The laser light and magnetic field stimulation results in additional increase of iron content, which allows to cover the total demand for iron in one meal a day. The amaranth contains comparable or higher amounts of magnesium than chocolate or cacao pulver (Łoś-Kuczera and Piekarska, 1988; Silverman *et al*., 2002; Starobat-Hermelin and Kozielec, 1997). Amaranth is a phosphorus-rich grain. Content of phosphorus in 100 g of amaranth seeds covers between 30 and 60% of a daily requirement for human (White and Broadley, 2005). Cereals such as wheat and barley contain between 0.7-0.9 g kg^{-1} of this mineral. 100 g of amaranth seeds cover in full the human demand for copper, manganese, and zinc (Salgueiro *et al.*, 2000; Uriu-Adams and Keen, 2005; White and Broadley, 2005).

Table 2 shows the fatty acid content of the amaranth seeds. No significant difference was observed in total fatty acids content. In all the seeds mono- and polyunsaturated acids dominated in all tested fats (69.6, 74.5, 74.4 and 72.6% respectively for control, laser stimulated, magnetic field stimulated and the seeds stimulated with both methods). It is in an agreement with the previously data (Becker, 1989).

The detailed analysis on saturated fatty acids shows the decrease in the levels of lauric (12:0), myristic (14:0) and palmitic (16:0) acids and the increase in stearic acid (18:0). Saturated fatty acids increase the risk of heart diseases. They also increase the level of LDL (bad cholesterol) therefore decrease in their level is highly demandable.

Although the method used does not allow for estimation of the influence of stimulation methods on the content of longer chain fatty acids, the detectable amounts of arachidic and behenic acid in the samples stimulated with magnetic field suggest that the levels of these amino-acids probably increase. Although C24:0 (lignoceric) and C24:1 (nervonic) fatty acids were investigated their levels were below the detection ability of the method used.

Essential fatty acids are necessary fats derived from linoleic and oleic acids that human cannot synthesize and which must be obtained through diet. Upon electromagnetical stimulation the level of mono- and polyunsaturated fatty acids increases. Although the increase of polyunsaturated fatty acids is not significant (6% for magnetic field stimulated sample while the admissible error was *ca* 5%), one should put the attention to the fact that the mono-unsaturated fatty acids level increased by up to 12% for the laser stimulated sample as compared to control. Therefore an increase in the level of these fatty acids is highly demandable from the nutritional point of view. Both monounsaturated as well as polyunsaturated 'cis' fatty acids can be beneficial in cell membranes by preventing the tight packing of fatty acids in membranes – thereby making the membranes more fluid. Membrane fluidity is important for optimal function of most cells in the body. But membrane fluidity is especially important on portions of cells that act as receptors for hormones or neurotransmitters (Clandinin *et al*., 1991).

CONCLUSIONS

1. The presowing electromagnetical stimulating methods cause the significant change in the mineral composition of the amaranth seeds. In particular, a significant decrease of the levels of potassium (from 8.92 for control sample to between 5.42 and 6.47 g kg^{-1} for electromagnetical stimulated samples), calcium (from 2.30 to 1.19-1.61 g kg^{-1}) magnesium (from 4.27 to 2.99-3.63 g kg^{-1}), sodium (from 76.40 to 25.07-40.06 g kg^{-1}), copper (from 10.97 to 7.20- 8.74 g kg^{-1}) and manganese (from 54.50 to 30.77-41.93 g kg^{-1}) ($P \leq 0.01$) was observed. The stimulated seeds contained comparable amount of phosphorus (between 1.15 and 1.27 g kg^{-1}). Interestingly, stimulation with both electromagnetical methods resulted in significant increase in the level of zinc and over two-fold increase in iron content (from 85.37 to 180.87 mg kg^{-1}) in the seeds as compared to control.

2. The electromagnetical stimulation of amaranth seeds resulted in the increase of essential fatty acids and the decrease in most of the saturated fatty acids which is highly demandable from the point of healthy nutrition.

3. Usage of pre-sawing electromagnetical stimulation methods can be a non-invasive, successful tool in modification of the chemical composition of the amaranth seeds.

T a b l e 2. Fatty acids content of amaranth seeds in g/100 g of crude fat

Fatty acid	C	L	F	L+F
	(g 100 g^{-1})			
12:0	0.49	0.06	0.06	0.03
14:0	0.24	0.15	0.13	0.17
14:1	Tr.	0.01	Tr.	0.03
16:0	17.02	13.81	13.95	15.18
16:1	0.09	0.10	0.09	0.09
18:0	2.12	2.42	2.48	2.32
18:1 cis	19.13	20.78	20.63	19.28
18:2	24.84	25.9	26.14	25.92
18:3	1.29	1.05	1.62	1.47
20:0	Tr.	Tr.	0.02	Tr.
20:1	Tr.	0.02	Tr.	Tr.
20:2	0.10	0.17	0.16	0.10
20:3	Tr.	Tr.	0.02	Tr.
20:4	0.10	Tr.	Tr.	Tr.
22:0	Tr.	Tr.	0.06	Tr.
22:1	Tr.	0.04	Tr.	Tr.
Saturated	19.87	16.44	16.70	17.70
Monounsaturated	19.22	20.95	20.72	19.40
Polyunsaturated	26.33	27.12	27.94	27.49
Fatty acids-total	65.42	64.51	65.36	64.59

Explanation as in Table 1. Fatty acid:

12:0 - dodecanoic (lauric)

14:0 - *n*-tetradecanoic (myristic)

14:1 - *cis*-9-tetradecenoic (oleomyristic)

16:0 - *n*-heksadecanoic (palmitic)

16:1 - *cis*-9-hexadecenoic (palmitoleic)

18:0 - *n*-octadecanoic (stearic)

18:1 *cis* - *cis*-9-octadecenoic (oleic)

18:2 - *cis, cis*-9,12-octadecadienoic (linoleic)

18:3 *alpha* - *cis,cis,cis* - 9,12,15-octadekatrienic (alpha-linoleic)

18:3 *gamma* - *cis,cis,cis*-6,9,12 octadekatrienic (gamma-linoleic)

20:0 - *n*-eicosanoic (arachidic)

20:1 - *cis*-5 - eicosenoic (eicosenoic)

20:2 - *cis,cis*-8,11-eicosadienoic

20:3 - *cis,cis,cis*-8,11,14- eicosatrienoic

20:4 - *cis,cis,cis,cis*-5,8,11,14-eicosatetraenoic

22:0 - *n*-docosanoic (behenic)

22:1 - *cis*-13- docosenoic (erucic)

Tr. - trace (<0.01 g 100 g^{-1} crude fat)

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