Change in the Profile of Fatty Acids with a Very Long Chain under the Influence of the Microwave Electromagnetic Field and Drought

Ekaterina P. KONDRATENKO

Ph.D. (in Agriculture)

Professor

Kuzbass State Agricultural Academy

5, Markovtsev Str., Kemerovo, 650056, Russia

Olga M. SOBOLEVA

Ph.D. (in Biology)

Associate Professor

Kemerovo State Medical University

22a, Voroshilov Str., Kemerovo, 650056, Russia

Andrey S. SUKHIKH

Ph.D. (in Pharmaceutics)

Senior Researcher

Kemerovo State Medical University

22a, Voroshilov Str., Kemerovo, 650056, Russia

Marina G. KURBANOVA

Ph.D. (in Engineering)

Professor

Kuzbass State Agricultural Academy

5, Markovtsev Str., Kemerovo, 650056, Russia

Abstract

Features are considered of changing the profile of very long chain fatty acids (VLCFA) in the barley seedling composition in connection with the impact of two abiotic factors: microwave electromagnetic field (EMF) and drought. Studies were carried out on monthly seedlings of barley grown in a soil mixture of peat and sand under laboratory conditions. The experimental chime included six options: 1) control, without microwave processing, normal humidification; 2) EMF with a power of 0.42 kW, a frequency of 2.45 GHz, with an exposure of 11 seconds, normal humidification; 3) EMF with a power of 0.70 kW, a frequency of 2.45 GHz, with an exposure of 11

seconds, normal humidification; 4-6) the same options, but grown in conditions of water scarcity. The content of VLCFA was determined by mass spectrometry. The combined effect of EMF and water deficiency changes the profile of very long-chain fatty acids, which is expressed in a significant increase in Erucic acid in all variants. The action of EMF of 0.42 kW power during the cultivation of seedlings under normal humidification itself in an increase in the content of Erucic acid by 1.6 times, and of 0.70 kW power - by 1.9 times. Increased content of Arachidic and Behenic fatty acids. Changes in the profile of VLCFA in leaves under the influence of water stress and EMF are associated with the activation of the main adaptive systems in the body of seedlings.

Keywords: very long chain fatty acids; fatty acid composition; seedlings; barley; microwave electromagnetic field; drought.

Introduction

According to (Bettaieb et al., 2009), very long chain fatty acids (VLCFA) are structurally diverse molecules with a hydrocarbon chain having from 20 to 28 or more carbon atoms. In plant cells, both saturated VLCFA and with varying degrees of unsaturation ones are found. These fatty acids are indispensable participants in many biological processes that cannot occur through the usual shorter (C14-18) aliphatic chains. VLCFA formed in plants are components or precursors of numerous specialized metabolites synthesized in individual cell types.

Most studies report a low prevalence of these molecules in plant organisms. Despite the fact that they are found in small quantities in plant cells, very long-chain fatty acids perform important functions in the development processes. When their synthesis is disrupted at the genetic level, marked phenotypic effects are noted, which range from severe growth retardation to embryonic lethality.

Recently, more data have been accumulated that indicate the potential role of VLCFA as signaling molecules in controlling both biotic and abiotic stress both at the intracellular and extracellular levels (De Bigault Du Granrut, Cacas, 2016).

From a theoretical and practical point of view, it is important to study the signal system of plants that accompanies the development of a response to stress – for example, water deficit. Plants have a whole complex of different signaling molecules, which include fatty acids.

For a long time, the study of the signaling functions of fatty acids remains an important section of biology due to the diversity and importance of the functions performed by these compounds (Savchenko, Dehesh, 2014). Despite numerous important discoveries in this area, many plant regulatory mechanisms related to fatty acids remain poorly understood and require further study.

The study of issues related to the functions of fatty acids in the adaptation of plants to biotic and abiotic stress is an urgent task of modern biology and has both theoretical and practical significance.

Abiotic stresses include light, drought, salinity, heat, cold, waterlogging, nutrient deficiency and other abiotic environmental conditions, which are the main limiting factors affecting plant growth and development, and often lead to lower crop yields.

Fatty acids, which are part of lipids, serve as the main structural components of the cell membranes of a living cell. Structural features of fatty acids (the number of carbon atoms in the chain and the degree of unsaturation) determine their participation in various biological processes. At the same time, fatty acids are not passive building blocks, but, on the contrary, they are active participants and regulators of a whole complex of important biological processes. They form lipid-protein microdomains, determine the orientation and interaction of proteins. The most important physical and chemical properties of biological membranes, such as fluidity and permittivity, are determined by two important factors. The endogenous factor can be attributed to the fatty acid composition of the membrane. However, environmental conditions are no less important. Together, these two factors change the fluidity and permittivity of cell membranes.

The membrane fluidity directly depends on the proportion of unsaturated fatty acids while increasing the number of double bonds and the length of the hydrocarbon chain reduces the melting point of the corresponding fatty acid which leads to an increase in the plasticity of the lipid bilayer. This plays a significant role in the molecular mechanism of cell adaptation to temperature stress.

In turn, these properties of membranes influence the course of vital membrane and intracellular processes: the regulation of gene expression and the formation of adaptive responses to environmental stressors. Free fatty acids, which are not part of the membrane lipids, also act as active regulators of many processes in a living cell, cause inhibition of electronic transport in photosystem II, and activate mitochondrial ion channels.

In plants, the effect of fatty acids and their metabolites on the course of intracellular processes is especially pronounced under stressful conditions. Minor changes in the fatty acid composition of membrane lipids lead to changes in the metabolism and activity of signaling pathways, which affects the course of physiological processes, primarily the formation of responses to environmental stressors.

In addition to the signaling function, the VLCFA obtain another one - structural function - these substances are both components and precursors of epicuticular wax. This fact confirms that VLCFA are of great importance in drought tolerance of plants. Under the influence of water stress in plants, the formation of this hydrophobic layer with the participation of VLCFA occurs

(Zhu, Xiong, 2013). Cuticular wax plays an important role in the resistance of plants to abiotic and biotic stresses (Zhang et al., 2017). Free VLCFA can form esters with various hydroxyl groups and, in particular, with alcohols, as occurs in the formation of waxes. In this case, VLCFA are essential, since they serve as substrates for enzymes that synthesize these waxes.

In recent years, there has been an increase in the number of extreme climate events (droughts, frosts, floods, warm winters). The dependence of agriculture on climate, if we are to judge by absolute losses, has increased significantly during this time. Crop losses due to adverse weather conditions in some years can reach 50-65% relative to the maximum possible. At the same time, water scarcity remains one of the leading stressors, causing enormous damage to agriculture, especially in areas of risky agriculture. Such regions include a significant share of all agricultural territories of the Russian Federation.

Drought, being one of the main types of abiotic stress, poses huge threats to global food security and the health of terrestrial ecosystems, limiting plant growth and crop productivity. Stress from water scarcity, which leads to a decrease in crop yields worldwide, is considered as the main reason affecting the growth and productivity of plants. Globally, crop losses from drought will amount up to 30% by 2025 compared to the current crop yields of previous years (Zhang, 2011).

Unlike animals, plants must withstand abiotic stresses without the ability to leave an unfavorable habitat. To survive under dry stress conditions, plants have developed complex mechanisms through integrated molecular and cellular reactions to achieve physical adaptation, such as a deep root system, effective stomata structure and regulation of leaf morphology, thickening of cuticular wax and cutinization of leaf surface (Zhang et al., 2017).

Cuticular wax provides a significant barrier to protecting plants from water stress - drought. Arid stress strongly affects the biosynthesis and fatty acid composition of cuticular wax. Field physiological studies have shown that wheat and barley yields are positively associated with cuticular wax, especially in drought conditions (González, Ayerbe, 2010).

The fatty acid composition also changes under the influence of other factors, for example, after the action of an electromagnetic field of ultrahigh frequency (microwave EMF). In the leaves and roots of barley seedlings developing from seeds after microwave treatment, Palmitic, Stearic, Behenic and Lignoceric acids are predominant among the limiting fatty acids, Palmitic and Stearic acids are found in the endosperm and casing of the caryopsis. Oleic and Linoleic acids prevail among unsaturated fatty acids in all organs of the seedling (Soboleva et al., 2019).

An analysis of the changes in fatty acids occurring in different organs of the seedling showed that in the sprouts, endosperm and membrane under the influence of the microwave electromagnetic field

there is a decrease in the content of saturated fatty acids and, accordingly, an increase in unsaturated fatty acids. The opposite tendency was revealed in the roots - an increase in the amount of saturated and a decrease in unsaturated fatty acids. It was established that changes in the fatty acid composition of lipids and the level of activity of enzymes such as ω 9-, ω 6- and ω 3-desaturases under the influence of an electromagnetic field of ultrahigh frequency also have differences in the organs of the seedling. The microwave electromagnetic field affects the biosynthesis of fatty acids, lipids and activation of the synthesis of enzymes involved in this process (Kondratenko et al., 2017).

Long-distance transport of fatty acids is impossible in plants. However, each cell of the plant organism contains fatty acids in the composition of membrane lipids. This means that each cell must have fatty acid biosynthesis enzymes. The synthesis of *de novo* fatty acids always occurs in plastids. According to recent data, a significant amount of lipid metabolism enzymes is located in plasmalemma.

Elongation of the hydrocarbon chain occurs at the endoplasmic reticulum using elongases, and the introduction of additional double bonds - desaturases. Stages of biosynthesis of fatty acids occur with the help of multi-enzyme complexes.

The **purpose** of the research was to study the change in the profile of the VLCFA in the leaves of barley seedlings under the influence of the microwave electromagnetic field and water deficit.

Methods

The object of research was seedlings of spring barley variety Nikita. The experiment scheme included six options:

- 1) control option, without microwave processing, normal hydration;
- 2) microwave processing with a power of 0.42 kW, a frequency of 2.45 GHz, with an exposure of 11 seconds, normal humidification;
- 3) microwave processing with a power of 0.70 kW, a frequency of 2.45 GHz, with an exposure of 11 seconds, normal humidification;
- 4) control option without microwave processing, drought;
- 5) microwave processing with a power of 0.42 kW, a frequency of 2.45 GHz, with an exposure of 11 seconds, drought;
- 6) microwave processing with a power of 0.70 kW, a frequency of 2.45 GHz, with an exposure of 11 seconds, drought.

After microwave treatment of dry seeds in the prescribed mode, sowing was carried out in pots with a mixture of highmoor neutralized peat (pH 5.5) and sand in a ratio of 5:1. Plants were grown on the soil in individual pots, watered with 50 milliliters of water every 2-3 days for three weeks. After that, watering was stopped. At the age of 1 month, the aerial mass of seedlings was extracted with a mixture of chloroform:*n*-hexane according to the procedure (Zakharova, Sukhikh, 2015). Then an aliquot of the sample was blown off with argon almost to dryness. To the residue it was added 500 µl of a 3% solution of H₂SO₄ in methanol and 100 µl of toluene. An internal standard (5 µg of methylundecanoate) was added to the resulting solution. Then the sample was heated at 90 °C for one hour. Next, extraction was carried out with 700 µl of hexane (in three portions). The volume of the selected hexane fraction was concentrated by blowing off the solvent to a volume of about 50 µl. The resulting sample containing methyl ester fatty acids was used for analysis. The analysis was carried out on a mass spectrometer Agilent 7000B (USA). Sample volume was 2 µl, injection was done without dividing the flow. Column: ZB-WAX, 30 m x 0.25 mm x 0.25 µm. Chromatography conditions: Oven Program at 100 °C for 0 min, then heating at a rate of 7 °C/min up to 260 °C - 10 min; flow rate - 1.2 ml / min. Identification was carried out by mass spectra (mass spectra library - NIST 02.L) and retention indices. The calculation of the mass content of fatty acid methyl esters was carried out relative to the known amount of methyl undecanoate (internal standard). Calibration was performed by using standard samples (Sigma-Aldrich), consisting of chains of various lengths and saturations (8:0, 16:0, 8:1, 20:4, 22:6). All measurements were performed in triplicate biological and triplicate analytical replicates; the tables show the average data. The results obtained were processed statistically - the significance of differences compared with the control was found by the F-criterion at a significance level of 0.05.

Results

As a result of the scientific experiments on the effect of ultra-high frequency electromagnetic fields (microwave EMF) and water deficiency on the processes of quantitative changes in the content of VLCFA in the cells of barley seedlings, it was established that under the influence of electromagnetic fields and drought, deep biochemical changes in the fatty acid composition occur (Figure 1).

Figure 1. VLCFA content in the leaves of barley seedlings under the influence of microwave and drought, %

1) control, without microwave processing, normal hydration; 2) microwave processing with a power of 0.42 kW, a frequency of 2.45 GHz, with an exposure of 11 seconds, normal humidification; 3) microwave processing with a power of 0.70 kW, a frequency of 2.45 GHz, with an exposure of 11 seconds, normal humidification; 4) control, without microwave processing, drought; 5) microwave processing with a power of 0.42 kW, a frequency of 2.45 GHz, with an exposure of 11 seconds, drought; 6) microwave processing with a power of 0.70 kW, a frequency of 2.45 GHz, with an exposure of 11 seconds, drought.

When clarifying the combined effect of drought and EMF of medium rate and high power on changes in the VLCFA profile, it was found that such an effect of two abiotic factors led to a decrease in Erucic acid by 1.4 times and 1.1 times, respectively, compared with the control option (drought), Lignoceric – by 1.2 times and 1.3 times. Meanwhile, the negative effects of erucic acid on the human body and some farm animals are known. Myocardial lipidosis was identified as the critical effect for chronic exposure to erucic acid. However, there is evidence of a wider range of organ (such as changes in the liver, kidneys, skeletal muscle, adrenals and testis weight) lesions that are formed under the influence of this fatty acid (EFSA Panel on Contaminants in the Food Chain (CONTAM et al., 2016).

The mass fraction of Hexacosanoic acid increased under the influence of EMF of average power and drought by 2.95%, remaining at the level with the control option with increasing power of EMF.

With an increase in EMF power and drought, this combined effect leads to an increase in Arachidic and Behenic fatty acids in the leaves of barley seedlings.

Discussion

An analysis of the results showed that five VLCFA are synthesized in the leaves of barley seedlings, of which four are limiting: Arachidic C 20:0, Behenic C 22:0, Lignoceric C 24:0, Hexacosanoic C 26:0 and one unsaturated Erucic C 22:1. All of them have from C 20 to C 26 carbon atoms.

In a comparative analysis, the influence of water and drought on the VLCFA content revealed that under the influence of water deficit the mass fraction of unsaturated Erucic and marginal Lignoceric acids increases significantly. The difference in these fatty acids with the control variant, which grew under conditions of normal moisture, was 1.22 times and 34.07%, respectively. Thus, abiotic stress such as drought during the growth and development of barley seedlings leads to a pronounced increase in the mass fraction of very long chain fatty acids.

The obtained experimental data suggest that the mechanisms of EMF and drought action with the determined characteristics realize themselves through the activation of abiotic stress, the development of which is associated with an increase in the production of VLCFA. The current studies do not contradict the data obtained in other experiments. Thus, the content of Erucic acid in the composition of rapeseed oil increases with drought (Moradbeigi et al., 2019).

If there is a water deficit during the growth and development of barley seedlings, there were a decrease in the leaves of the mass fraction of VLCFA with an even number of carbon atoms C20:0, C22:0, C26:0 and an increase in C22:1 and C24:0 in the leaves.

The action of medium-power EMF had an impact on the accumulation of VLCFA in the cells of the leaves of barley seedlings. An increase in C22:1 in 1.6 times and a decrease in the mass fraction of C20:0 and C22:0 in comparison with the control variant were found by 2.38% and 10.62%, respectively.

As a result of the experiment, it was found that, under the influence of magnetic fields after microwave power with high power without water deficiency, the content of Arachidic and Behenic acids significantly increases by 1.3 times, and Hexacosanoic acid decreases by 1.3 times compared

to the control option (without treatment). The content of Erucic and Lignoceric acids increases by 0.1%, and 1.4%, respectively.

Important is the fact that the action of the electromagnetic field does not in itself lead to a change in the profile of fatty acids – this has been proven in the microwave treatment of various vegetable oils. Only the active response of the plant as a living organism to the action of the abiotic factor leads to quantitative changes in the composition of fatty acids (Olabemiwo et al., 2014).

In experiments, it was found that drought leads to an increase in the production of Erucic acid (C22:1) in the leaves of barley seedlings by 1.6 times, and the effect of EMF of average power is 1.9 times compared to the control option.

Water stress affects the content of fatty acids in the direction of increasing their content, as well as the appearance of new fatty acids. However, in different objects of research, in separate experiments on the same object, as well as in different fractions of lipids, even the same organ of the plant often has to deal with the variability of their composition (Zhukov, 2018).

Conclusion

Under the influence of abiotic factors such as drought and electromagnetic field of ultrahigh frequency of medium and high power on the plant organism, a change in the profile of fatty acids with a very long chain is noted. A significant increase in Erucic acid was revealed in all variants of the experiment. At the same time, the effect of medium-sized electromagnetic fields during the cultivation of barley seedlings without water deficit relative to their cultivation under conditions of water shortage led to an increase in Erucic acid by 1.6 times.

When determining the content of Erucic acid in barley seedlings grown with a sufficient amount of water and with a water deficit, a significant increase in the production of barley seedlings of this acid by 2.2 times was also established. An increase in the content of Arachidic and Behenic fatty acids is also noted.

Thus, changes in the VLCFA profile in leaves under the influence of water stress and EMF are associated with the activation of the main adaptive systems in the body of barley seedlings. When exposed to the seeds of cereal EMF microwave and water deficiency, they respond to these abiotic factors with an active adaptive reaction, manifested in the fluctuations of certain fatty acids with a very long chain.

References

- Bettaieb, I., Zakhama, N., Wannes, W.A., Kchouk, M.E., Marzouk, B. (2009). Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition. *Scientia Horticulturae*, 120(2), 271-275. <https://doi.org/10.1016/j.scienta.2008.10.016>
- De Bigault Du Granrut, A., Cacas, J.L. (2016). How very-long-chain fatty acids could signal stressful conditions in plants? *Frontiers in plant science*, 7, 1490. <https://doi.org/10.3389/fpls.2016.01490>
- EFSA Panel on Contaminants in the Food Chain (CONTAM) et al. (2016). Erucic acid in feed and food. *EFSA Journal*, 14(11), e04593. <http://doi.org/10.2903/j.efsa.2016.4593>
- González, A., Ayerbe, L. (2010). Effect of terminal water stress on leaf epicuticular wax load, residual transpiration and grain yield in barley. *Euphytica*, 172, 341-349. <https://doi.org/10.1007/s10681-009-0027-0>
- Kondratenko, E.P., Soboleva, O.M., Sukhikh, A.S. (2017). Influence of microwave electromagnetic field on the fatty acid composition of seedlings *Hordeum sativum*. *Khimiya Rastitel'nogo Syr'ya*, 3, 93-99. <https://doi.org/10.14258/jcprm.2017031792> (in Russian)
- Moradbeigi, L., Gholami, A., Shiranirad, A.H., Abbasdokht, H., Asghari, H.R. (2019). Effect of Drought Stress and Delay Cultivation on Grain Yield, Oil Yield and Fatty Acids Composition in Canola. *Journal of agricultural science (University of Tabriz)*, 29(2), 135-151.
- Olabemiwo, O.M., Esan, A.O., Adepoju, A.J. Omodara, N.B. (2014). Effect of microwave heating on fatty acid profiles of three nigerian vegetable oils. *Journal of Applied Chemistry*, 7(4), 51-54.
- Savchenko, T., Dehesh, K. (2014). Drought stress modulates oxylipin signature by eliciting 12-OPDA as a potent regulator of stomatal aperture. *Plant Signaling and Behavior*, 9(2), 1151-1160. <https://doi.org/10.4161/psb.28304>
- Soboleva, O.M., Kondratenko, E.P., Sukhikh, A.S. (2019). Profile of higher fatty acids of barley seedlings after treatment with electromagnetic waves of the microwave range. *Bulletin of higher educational institutions. Volga region. Natural Sciences*, 4, 5-15. <https://doi.org/10.21685/2307-9150-2019-4-1> (in Russian)
- Zakharova, Y.V., Sukhikh A.S. (2015). Fatty acids of the cell walls of bifido-bacteria with different hydrophobicity. *Bulletin of the Orenburg Scientific Center of the Ural Branch of the Russian*

Academy of Sciences, 3, 1-12 Retrieved from:
<http://elmag.uran.ru:9673/magazine/Numbers/2015-3/Articles/ZJV-SAS-2015-3.pdf>
(in Russian)

- Zhang, J. (2011). China's success in increasing per capita food production. *Journal of Experimental Botany*, 62(11), 3707-3711. <https://doi.org/10.1093/jxb/err132>
- Zhang, X., Lu, X., Chen, G., Chen, Z. (2017). Molecular and evolutionary mechanisms of cuticular wax for plant drought tolerance. *Frontiers In Plant Science*, 8, 1-12. <https://doi.org/10.3389/fpls.2017.00621>
- Zhu, X., Xiong, L. (2013). Putative megaenzyme DWA1 plays essential roles in drought resistance by regulating stress-induced wax deposition in rice. *Proc. Natl. Acad. Sci. U.S.A.*, 110(44), 17790-17795. <https://doi.org/10.1073/pnas.1316412110>
- Zhukov, A.V. (2018). Very long chain fatty acids in plant membrane lipids. *Plant physiology*, 65(6), 784-800. <http://doi.org/10.1134/S1021443718050187>