Abstracts of peer-reviewed publications

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1. **Kuzmanova M.,** S.Ivanov M.Baldgiiska, B.Videlov, V.Nankova, N.Neshev, S.Alexandrov, M.Markov (1993) Millimeter waves protect gamma-irradiated rats. in: *Electricity and Magnetism in Biology and Medicine*, M. Blank, Ed., San Francisco Press, Inc., 596-598.
ISBN-13: 978-0911302677 ISBN-10: 0911302670

Abstract

Millimeter waves (53.57 GHz, 1 mW/cm^2) and gamma-radiation (6 Gy) were applied to white male rats (Wistar, 170 g). Nonionizing radiation was applied to the right forelimb by a Г4-142 generator. Gamma-rays irradiated the whole body of the animals by use of IGUR device. The amount of ATP and electrophoretic mobility of erythrosytes, as well as the spleen weight and spleen cariocyte counts, were evaluated 3, 14 and 21 days following exposure. It was found that millimeter waves substantially reduced some of the most deleterious effects of gamma radiation, especially when they were applied prior to exposure to ionizing radiation.

2. Traikov L.L., M.S. Markov, **M.A. Kuzmanova**, S.P. Ivanov, 1994, Use of lectins as indicators for magnetic field action on erythrocyte membranes, *Reviews on Environmental Health*, 10(3-4): 243-246. DOI:10.1515/REVEH.1994.10.3-4.243 Corpus ID: 28219203

Abstract

The effects of magnetic fields on biological membranes in general and on the red blood cell membrane in particular have been studied intensively in the last two decades. A variety of methods for evaluation of magnetic field action on the structure and function of biological membranes has been used. It has been suggested /1/ that the cell membrane can be considered as one of the primary targets affected by magnetic field exposure.

3. Traikov L.L., **M.A. Kuzmanova**, S.P. Ivanov, M.S. Markov, 1994, Effect of static magnetic field on lectin binding to erythrocyte membrane, *Bioelectrochem. & Bioenerget.*, 35(1-2): 49-52.
(cera *Bioelectrochemistry*) https://doi.org/10.1016/0302-4598(94)87010-1

Abstract

It is accepted that magnetic field bioeffects depend strongly on the physiological state of biomembranes as well as on the presence of certain physical and/or chemical agents, including lectins, which can initiate an agglutination. This study explores the advantages of immunochemical and biophysical methods for the evaluation of changes which occur in the erythrocyte membrane under the action of a magnetic field. The possibility of modification of the antigen-antibody reaction by the application of magnetic fields and the ways in which this modification affects both the ATP content and electrophoretic mobility of red blood cells are a subject of investigation. The results show that a static magnetic field of 5 mT changes significantly the binding properties of lectin as the time necessary for complete binding increases to 50-60 min. It appears that this magnetic field alters the processes of lectin binding to the specific binding sites on the surface of the erythrocyte membrane. The rate and extent of lectin binding may be an indicator of changes in the glycoprotein complex.

4. **Kuzmanova M.**, S. Ivanov, V. Nankova, M. Markov, 1994, Effect of extremely high frequency electromagnetic fields on electrophoretic mobility and ATP content in rat erythrocytes, *Bioelectrochem. & Bioenerget*., 35(1-2): 53-56. IF 0.940 за 1994 г. (сега *Bioelectrochemistry*) https://doi.org/10.1016/0302-4598(94)87011-X

Abstract

Millimeter waves (53.57 GHz, 1 mW cm^{-2}) were applied to right forelimbs of white male rats (Wistar, 180–200 g). The amount of ATP, and the electrophoretic mobility of erythrocytes in six different variants were evaluated following different exposure conditions. It was found that millimeter waves altered substantially the electrophoretic mobility and ATP content in rat erythrocytes. Changes after 5 days of exposure were the most significant.

5. **Kuzmanova M.**, S. Ivanov, V. Nankova, M. Markov, 1995, Extremely high frequency electromagnetic fields effects on cell membranes, In: *Charge and Field Effects in Biosystems - 4*, Proceedings of the 1994 International Symposium, Virgina Commonwealth University, Richmond, Virginia, 20–24 June, 1994. M.J. Allen, S.F. Cleary, A.E. Sowers (Eds.), World Scientific, p. 243-254. ISBN: 978-981-4550-35-2 (ebook) https://doi.org/10.1142/2492

Abstract

Extremely high frequency (30–300 GHz) electromagnetic fields have an increasing application as a diagnostic and therapeutic method. Millimeter waves are considered basically capable of modifying the existing processes and systems for generation, transmission and reception of signals for information and regulation. This study evaluates the effects of extremely high frequency electromagnetic fields on erythrocyte membrane. Millimeter waves (53.53 GHz, 1 mW/cm^2) were applied to the right forelimb of white male rats (180–200 g). The amount of ATP and electrophoretic mobility (EPM) of erythrocytes in six groups of animals was evaluated following different exposure conditions. It was found that millimeter waves substantially altered both ATP content and EPM of erythrocytes within the first 5 days of irradiation, and a tendency of adaptation toward applied factor was observed when exposure to high frequency field is further prolonged. A surprising increase was found in red blood cells from animals which had been exposed to millimeter waves for 10 days, but the blood for analysis was taken 10 days later. The dynamics of EPM shows statistically significant changes in all exposure conditions. The observed changes can be considered as a result of modifications in the glycolytic processes which reflect in the alteration of the activity of a whole range of enzymes. These changes reflect the alterations of the cell surface charge (in general) and redistribution of charged molecules and/or domain over the cell membrane surface (in particular).

6. Traikov L., **M. Kuzmanova**, S. Ivanov, M. Markov, 1995, Static magnetic field effects basic glycoprotein complex of the erythrocyte membrane, In: *Charge and Field Effects in Biosystems – 4*, Proceedings of the 1994 International Symposium, Virgina Commonwealth University, Richmond, Virginia, 20–24 June, 1994. M.J. Allen, S.F. Cleary, A.E. Sowers (Eds.), World Scientific, p. 334-348. ISBN: 978-981-4550-35-2 (ebook) https://doi.org/10.1142/2492

Abstract

It has been shown that magnetic field initiated alterations in the membrane and cell activity strongly depend on the physiological state of biomembranes as well as on the presence of certain chemical agents. This study explores the advantages of immunochemical and biophysical methods for evaluation of the changes which occur in the erythrocyte membrane under static magnetic field action. The lectin-binding assay was based on 50–60 min incubation of suspension of human erythrocytes with lectin (phytochemagglutinin) and exposure to static magnetic field in a 1–90 mT range. The ATP content, transmembrane potential and electrophoretic mobility (EPM) of human red blood cells were investigated. It was found that the magnetic field alone initiated the most significant alterations of both the ATP content and of the EPM. The ATP and EPM changes strongly depended on duration of exposure: 30–45 min exposure time appeared as a specific time window. The static magnetic field of 5 mT significantly changed the binding properties of lectin as the time necessary for complete binding increased to 50–60 min. The alterations of membrane characteristics suggests that magnetic field affects the membrane surface charge distribution and alters the binding properties of antigen determinants situated on the membrane surface. The transmembrane potential also change under magnetic field action. It appears that the static magnetic field alters the process of lectin binding to the specific binding sites on the surface of the erythrocyte membrane.

7. **Kouzmanova M.**, S. Ivanov, V. Nankova, L. Traykov, 1997, Effects of ionizing and nonionizing radiation on rat hemopoiesis, *Electro- and Magnetobiology* 16(2): 161-168. IF 0.729 (cera *J. Electromagnetic Biology and Medicine*) https://doi.org/10.3109/15368379709009841 https://www.tandfonline.com/doi/abs/10.3109/15368379709009841

Abstract

Alterations of the marrow cellularity in male Wistar rats under the combined action of millimeter waves (MMW) and gamma rays were studied. MMW with wavelength of 5.6 mm (frequency 53.53 GHz) or 7.1 mm (42.19 GHz) were applied to the right hindleg shank of the animal for 20 min daily for 10 days using a Yav'-1 generator (Russia). Two of the test groups were also irradiated with gamma rays (6 Gy) immediately following the last treatment with MMW. Nucleus-containing cells in the marrow from a 1-cm-long piece of thigh bone were counted on days 3, 7, 14, 21, and 30 after the last treatment with MMW or after the gamma irradiation. The treatment of the animals with 5.6 mm electromagnetic waves (EMW) only showed an increase of marrow cellularity compared to the control group after day 14 until the end of the investigation period. An increase in marrow cellularity after exposure to 7.1 mm EMW was observed at the seventh day following exposure. Exposure of the rats to MMW prior to gamma irradiation reduced the radiation damage of hemopoiesis in the early postirradiation period. The exposure to MMW increased the quantity of marrow karyocytes after the ionizing exposure and preserved the proliferating capability of the stem cells and their active differentiation. The results of this study showed that bone marrow cellularity returned to control values on day 7 after gamma irradiation, and greater effect was achieved using 7.1 mm EMW.

8. **Kouzmanova M.**, S. Ivanov, V. Nankova, L. Traikov, 1997, Effects of millimeter waves and γ-rays on the spleen weight and cellularity, *Annuaire de l'Universite de Sofia*, 87-89(3): 79-84.

Abstract

This study examines the combined effects of millimeter electromagnetic waves (MMW, wavelength 5.6 mm, frequency 53.53 GHz, and 7.1 mm, 42.19 GHz) and gamma-rays (6 Gy) on the spleen weight and on the number of spleenocariocytes in rats (Wistar, 170–190 g). The spleen weight showed an increase after the treatment of animals with 5.6 mm EMW but the differences were statistically insignificant. Exposure to 5.6 mm EMW had no effect on the number of spleenocariocytes. The treatment with 7.1 mm EMW didn't significantly influence the spleen weight also. However it decreased the spleen cellularity on the 3rd day. For the rest of examined periods the number of spleenocariocytes was close to the control one.

The treatment of the animals with MMW prior to gamma-irradiation corrected the radiation-induced alterations, especially in the last postradiation periods.

9. Traikov L.L., **M.A. Kuzmanova**, M.S.Markov, 1999, Combined action of static magnetic field and temperature on surface electric charge of the erythrocyte membrane, in: *Electricity and Magnetism in Biology and Medicine*, F. Bersani (Ed.), Kluwer Academic/Plenum Publishers, p. 561-563. ISBN 978-1-4613-7108-0 ISBN 978-1-4615-4867-6 (e-book)

DOI 10.1007/978-1-4615-4867-6 https://www.springer.com/gp/book/9780306460418

Introduction (there is no Abstract)

Several studies on the identification and characterization of the membrane surface electrical charge and membrane transport modification under magnetic field (MF) action were published during last decade. They attempted to elucidate physical or physicochemical parameters which are responsible for membrane alterations[1-4].

One of the most important physiological properties of the erythrocytes is the capability of changing on membrane activity in accordance with variations of the temperatures in the surrounding tissues/medium. The response to the temperature changes is multilateral and connected with a very complicated cascade of events. The alterations of membrane properties are better seen at temperatures corresponding to lipid phase transitions, which in tum reflect on a general reconstruction of lipid-protein interactions and redistribution in the membrane charge. This study was therefore designed to search for modification in the surface electric charge manifested in changes of the electrophoretic mobility (EPM) and band-3 channel properties, when static magnetic field was applied consequently and in combination with different temperatures (20°, 25°, 38°C).

10. **Kouzmanova M.**, S. Ivanov, 1999, Effects of millimeter waves on the concentration of ceruloplasmin in the blood plasma of gamma-irradiated rats, in: *Electricity and Magnetism in Biology and Medicine*, F. Bersani (Ed.), Kluwer Academic/Plenum Publishers, p. 645-647. Book ISBN 978-1-4613-7108-0 ISBN 978-1-4615-4867-6 (e-book)
DOI 10.1007/978-1-4615-4867-6 https://www.springer.com/gp/book/9780306460418

Abstract (Annotation)

Environmental factors can influence the character of the immune response, as well as the degree and the duration of the humoral immunity. In recent years the investigations of the effects of low intensity physical factors on an organism are of considerable interest, and in case of combined actions and additional burden on the immune system, especially.
https://link.springer.com/chapter/10.1007/978-1-4615-4867-6_153

11. **M. Kouzmanova**, M. Hristova, K. Vangelova, 2005, Alteration of erythrocyte electrophoretic mobility of operators in communication stations, *Annuaire de l'Universite de Sofia "St. Kliment Ohridski"*, 96(I): 215–219.

Abstract

The electrophoretic mobility (EPM) is a quantative biophysical indicator, which gives information about the electric charge on the cell surface. In clinical practice this charge is a marker for a number of diseases. This study was designed to investigate the changes in erythrocyte EPM of the operators in communication stations. The blood samples were taken from the workers at the beginning and after the end of the 24-h stay in the station. The EPM was estimated by using microelectrophoretic device Cytopherometer (Opton, Germany). The Student-Fisher's t-test was used to evaluate the statistically significant differences between the experimental groups. Erythrocyte EPM increases right after the end of the 24-h stay in the station. Statistically reliable alterations are detected between the same experimental subgroups in the different stations. They are probably a result from long-lasting exposure on EM radiation with various frequencies and intensities. The obtained results show that working for years in environment with EM radiation is connected with permanent alteration of the erythrocyte surface charge.

12. **M. Kouzmanova**, M. Hristova, 2005, Effects of *in vitro* microwave exposure on electrophoretic mobility of human erythrocytes, *Annuaire de l'Universite de Sofia "St. Kliment Ohridski"*, 96(I): 221 – 227.

Abstract

Considering the frequency dependence of the biological effects of electromagnetic fields (EMF), different models for interaction of high frequency EMF enegy and membrane components were suggested. The model for absorption of electromagnetic energy from bounded to biomolecules water is most probable. This study was designed to estimate the effects of 24 GHz microwaves on erythrocyte electrophoretic mobility (EPM) and their dependence on the suspension water content, on the exposure duration and on the time elapsed after irradiation. Human blood taken from health volunteers was used in the experiments. Erythrocyte suspensions with different water content (hematocrit 20, 40 or 60%) were exposed to microwaves for 15, 30, 45 and 60 min. The electromagnetic radiation was performed by K band traffic radar (24 GHz, minimal output power 5 mW). EPM was measured using microelectrophoretic device Cytopherometer (Opton, Germay)

0, 20, 40 and 60 min after the treatment. The Student-Fisher's t-test was used to estimate the statistically significant differences between the experimental groups. The microwave exposure causes alterations in the EPM that depend on the suspension water content, on the treatment duration, and on the time elapsed after irradiation. This corroborates hypothesis for importance of water in realization of high frequency EMF effects on living organisms.

13. **Kouzmanova M.**, G. Atanasova, N. Atanasov, S. Tasheva, 2007, Effects of *in vitro* exposure to GSM900 electromagnetic field on human erythrocytes, *Environmentalist*, 27: 423-428. (Cera: *Environment Systems and Decisions*) DOI 10.1007/s10669-007-9078-8

Abstract

High frequency electromagnetic fields (EMF) are widely used for transmitting of radio and TV signals, in wireless communications, etc. A huge number of people are exposed so the possible risk to human health from telecommunication technologies could be significant even if biological effects are slight. The study of the biological effects of RF EM radiation could contribute to better understanding of the possible health hazards. The levels of released hemoglobin serve as an indicator of hemolysis, caused by increased membrane fragility. This study was designed to investigate the alterations in hemoglobin release after in vitro exposure of human erythrocytes to GSM900 electromagnetic field. Erythrocyte suspensions with two different cell concentrations (hematocrit 20% and 40%) were exposed to EM radiation from GSM mobile phone (carrier frequency 902 MHz, 2 W output power in pulse) for 20 min in two different positions in relation to telephone antenna: Position 1 is in the centre of the major lobe of the azimuth antenna pattern and Position 2 is between major and back lobes. Alterations in hemolysis were registered 0, 10, 20, 30, 40, 50 and 60 min after the treatment. Hemolysis was determined by measuring the absorbance of hemoglobin at 413 nm in the supernatant obtained by centrifuging the suspensions. Hemolysis was expressed as hemoglobin concentration. Our data indicated decrease in the hemoglobin level in irradiated suspensions. The GSM900 EMF exposure probably stabilized erythrocyte membrane and caused reduction in the hemolysis depending on the EMF parameters, on the suspension water content (hematocrit) and on the time elapsed after irradiation.

14. Goltsev V., Tsimilli-Michael M., Chernev P., Zaharieva I., **Kouzmanova M.**, Strasser R.J. (2008) Electromagnetic frequency spectra of samples placed in a coil that senses the electromagnetic background field: Application for leaves, chloroplasts and molecules useful in photosynthesis. In.: J.F. Allen, E. Gantt, J.H. Golbeck & B. Osmond (eds.), *Photosynthesis. Energy from the Sun*: 14th International Congress on Photosynthesis, 591–596, Springer, Dordrecht. ISBN 978-1-4020-6707-5, ISBN 978-1-4020-6709-9 (e-book) https://www.springer.com/gp/book/9781402067075

Abstract

We are continuously surrounded by an electromagnetic field which can be picked up by a conducting coil and stored in a computer for further analysis. This field is an electromagnetic background noise. The aim of the current work is to clarify whether the presence of dissolved molecules/water systems placed in the detecting coil can be registered with physical methods and analyzed mathematically. We designed an experimental setup that included a sensitive coil and a signal amplifier in order to register the electromagnetic field with and without water solutions and living plant objects. The electrical signal was recorded by a 24-bit sound card of a laptop computer and was analyzed by the WaveLab 4.0 computer software. By a Fast Fourier Transformation the signal was translated into the frequency domain in the range from 20 Hz to

20 kHz. The resulted electromagnetic spectra were recorded in the presence of several photosynthetically active compounds (modifying the electron transfer reactions): the electron acceptors 2,6-dichlorophenol-indophenol, potassium ferricyanide and methylviologen, and the inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The difference spectra (the spectrum of the solution minus the spectrum of water) of all chemicals or thylakoid suspension show the existence of different maxima in the frequency range from 20 to 500 Hz which seem to be specific for the given material placed into the coil.

15. **Kouzmanova M.**, Dimitrova M., Dragolova D., Atanasova G., Atanasov N. (2009) Alterations in enzyme activities in leaves after exposure of *Plectranthus sp.* plants to 900 MHz electromagnetic field, *Biotechnology & Biotechnological Equipment* Special Edition, 23(2):611-615, ISSN 1310-2818.

Abstract

Accelerated and widespread use of different communication systems and modern electronic equipment increases exposure to radiofrequency electromagnetic fields (RF EMF) and raises serious concerns about the biological and health-related effects of RF radiation. Numerous studies document various biological effects of RF radiation. However, most of these findings derive from epidemiological, animal and *in vitro* studies while only a few investigate effects of RF radiation on plants. The purpose of our study was to investigate the alterations in enzyme activities in leaves after exposure of plants *Plectranthus sp.* to 900 MHz EMF and their dependence on the time elapsed after exposure. *Plectranthus* is the largest South African genus of plants belonging to the mint family (*Lamiaceae*). The ornamental herbaceous plant *Plectranthus sp.* was used as a model plant for investigating the effects of 900 MHz EMF. Plants were exposed whole body for 1 hour to EM radiation from GSM mobile phone (carrier frequency 902 MHz, 2 W pulse output power) at 20 cm distance from the antenna. Alterations in activity of isocitrate dehydrogenase, malate dehydrogenase and glucose-6-phosphate dehydrogenase in leaves were registered immediately after the end of the exposure and 1, 2 and 24 hours later. Irradiation of plants induced different alterations in enzyme activities depending on the time elapsed after irradiation. Immediately after exposure the activity of the three investigated enzymes decreased, but increased at 24th hour. In conclusion, the data provide evidence that plants perceive and respond to electromagnetic fields and are a good model to study the effects of mobile phone radiation.

16. Голцев В., И. Йорданов, М. Гурманова, **М. Кузманова**, Щ. Дамбов, С. Апостолова, Г. Савова, Р.Й. Страсер, 2010, Възможности на новия мултифункционален анализатор на ефективността на растенията за изследване на функционалното състояние на фотосинтетичния апарат, *Аграрни науки*, II (4): 15-26.

Vasilij Goltsev, Ivan Yordanov, Maria Gurmanova, **Margarita Kouzmanova**, Shteryan Dambov, Sonia Apîstîlova, Gergana Savova, Reto Jorg Strasser, 2010, Multifunctional Plant Efficiency Analyzer mPEA Used to Describe the Physiological States of the Photosynthetic Apparatus. *Agricultural Sciences*, II (4): 15-26. ISSN 1313-6577 DOI: 10.22620/agrisci.2010.04.002.

Resume

The rapid developments of molecular biological and molecular genetic techniques provide plant-growers with an instrument for target-oriented modifications of the plant genome and obtaining a large number of samples with different characteristics. Under these conditions a major problem for successful breeding work arises – a quick and efficient selection of promising samples with

the needed useful properties. Hansatech Instruments Ltd (Kings Lynn, UK) developed a new tool – mPEA (multifunctional Plant Efficiency Analyzer) allowing for fast and very informative sub-millisecond time resolution analysis (*in vivo* and *in situ*) of the functional status of the photosynthetic apparatus in plants. It is based on a simultaneous signal 16-bit resolution registration of the kinetic characteristics of prompt chlorophyll fluorescence emission, delayed chlorophyll fluorescence and modulated light scattering and reflection of the actinic incident light at 820 nm. Leaves from bean plants at different physiological states (control and decapitated after the appearance of the first trifoliate leaf) have been analyzed. The prompt fluorescence signal provides information about electron transport fluxes through Photosystem II and Photosystem I. The modulated reflection signal at 820 nm provides information about the activity of the donor and acceptor side of Photosystem I. The delayed fluorescence signals provide information about the oxygen evolving complex and the acceptor side of Photosystem II presenting structural information as rate constants, related to the whole photosynthetic apparatus.

17. **Кузманова М.**, М. Гурманова, С. Тинчева, В. Голцев, Г. Атанасова, Н. Атанасов, 2010, Влияние на GSM900 електромагнитни полета върху параметри на хлорофилната флуоресценция при културните растения пшеница, царевица и грах, *Аграрни науки*, II (4): 101-108.

Margarita Kouzmanova, Maria Gurmanova, Savina Tincheva, Vasilij Goltsev, Gabriela Atanasova, Nikolai Atanasov, 2010, Effects of GSM900 Electromagnetic Fields on some Parameters of Chlorophyll Fluorescence in Crop Plants Wheat, Maize and Peas. *Agricultural Sciences*, II (4): 101-108. ISSN 1313-6577 DOI: 10.22620/agrisci.2010.04.018.

Resume

For some years now the interest in the effects of mobile phones electromagnetic fields (EMF) on plants has been increasing steadily. The results show that plants respond to these EMFs as to a stress factor. Chlorophyll fluorescence is a sensitive and information-rich method for investigation of the effects of weak stressors of the photosynthetic process *in vivo* and *in situ*.

The aim of our study was to investigate the effects of different conditions of exposure to GSM900 EMF on some parameters of chlorophyll fluorescence in different crop plants: wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and peas (*Pisum sativum* L.).

The induction curves of prompt chlorophyll fluorescence were recorded with the fluorimeter Handy PEA (Plant Efficiency Analyser, Hansatech Instruments Ltd, UK). Some parameters of the JIP-test were analyzed.

The observed effects of 900 MHz EMF emitted by mobile phones depended on the plant species, duration of exposure to EMF and time elapsed after the end of exposure. Out of the two species of plants with different mechanisms of CO_2 fixation, maize (C4) showed greater sensitivity to 900 MHz EMF compared to wheat (C3) under the investigated exposure conditions.

Dark period exposure to GSM900 EMF, simulating radiation from a base station during rush hours, did not induce stress in pea plants estimated by prompt chlorophyll fluorescence parameters.

18. **Маргарита Кузманова**, Милена Димитрова, Даниела Драголова, Габриела Атанасова, Николай Атанасов, 2010, Влияние на продължително облъчване с GSM900 електромагнитни полета върху ензимната активност в листа на грах (*Pisum sativum* L.), *Аграрни науки*, II (4): 109-114.

Margarita Kouzmanova, Milena Dimitrova, Daniela Dragolova, Gabriela Atanassova, Nikolai Atanassov, 2010, Effects of Prolonged Exposure to GSM900 Electromagnetic Fields on Enzyme Activity in Leaves of Peas (*Pisum sativum* L.), *Agricultural Sciences*, II (4): 109-114.
ISSN 1313-6577 DOI: 10.22620/agrisci.2010.04.019.

Resume

Studies on the effects of radio frequency electromagnetic fields (RF EMF) on plants are few in number but the results suggest that plants respond to EMF used in mobile communications. The purpose of this work is to investigate the effects of prolonged base station EMF exposure on the enzyme activity in leaves of pea plants *Pisum sativum* L., variety RAN-1. Plants were divided into 3 groups of 5 plants: control, exposed and sham exposed. Exposed plants were irradiated for 14 days, 1 hour daily with a homogeneous electric component 42.6 V/m of 947.5 MHz continuous EMF simulating the emission of a BS during a rush hour. The activity of several respiratory enzymes (*isocitrat dehydrogenase, glucose-6-phosphate dehydrogenase* and *malic enzyme*) and peroxidases (*catalase, ascorbate peroxidase* and *guaiacol peroxidase*) was measured. The obtained results showed that prolonged exposure of pea plants during the dark period to homogeneous continuous electrical component of EMF radiation, simulating a base station during a rush hour, did not cause changes in the activity of respiratory and antioxidant enzymes in the leaves.

19. Goltsev V., M.Gurmanova, **M.Kouzmanova**, I.Yordanov, S.Qiang, A.Pentland, N.Wilson, S.Chen, I.Zaharieva, R.J.Strasser (2013) Symposium 05_03 Analysis of Dark Drops, Dark-Induced Changes in Chlorophyll Fluorescence during the Recording of the OJIP Transient. Tingyun Kuang, Congming Lu, Lixin Zhang (Eds), *Photosynthesis Research for Food, Fuel and the Future*, Proceedings of 15 International Congress on Photosynthesis, 2010, Beijing Springer Heidelberg New York Dordrecht London, p.179-183. ISBN 978-3-642-32033-0 ISBN 978-3-642-32034-7 (e-book) https://www.springer.com/gp/book/9783642320330

Abstract

In a search for a powerful and easy-to-handle instrument for investigation of photosynthetic energy and electron transfer reactions, the mPEA (Hansatech Instrument Ltd., King's Lynn, Norfolk, PE30 4NE, U.K.) was developed. The instrument can record simultaneously, in vivo, the photo-induced changes in prompt (PF) and delayed (DF) chlorophyll fluorescence and modulated infrared light reflection at 820 nm (MR820) using a protocol of alternating periods of illumination and darkness. In this way the standard OJIP induction transient of PF is modified by the dark intervals causing a decrease of the PF intensity during the dark periods (so called dark fluorescence drops, DD). The dependence of the relative DD on the redox level of Q_A was analyzed for different initial states of bean leaves. A strong linear correlation was found between the relative dark drops and the fraction of oxidized Q_A for the phase starting before the J level and going up to the P level of the PF induction transient. We propose that the experimentally measurable DDs offer a tool for *in vivo* quantification of the redox reactions of Q_A and Q_B during the fluorescence rise from F_0 to F_M.

20. Vasilij Goltsev, Ivelina Zaharieva, Petko Chernev, **Margarita Kouzmanova**, Hazem M. Kalaji, Ivan Yordanov, Vasilena Krasteva, Vladimir Alexandrov, Detelin Stefanov, Suleyman I. Allakhverdiev, Reto J. Strasser (2012) Drought-induced modifications of photosynthetic electron transport in intact leaves: Analysis and use of neural networks as a tool for a rapid non-invasive estimation. *Biochim. Biophys. Acta*, 1817: 1490-1498. doi:10.1016/j.bbabio.2012.04.018.

Abstract

Water deficit is one of the most important environmental factors limiting sustainable crop yields and it requires a reliable tool for fast and precise quantification. In this work we use simultaneously recorded signals of photoinduced prompt fluorescence (PF) and delayed fluorescence (DF) as well as modulated reflection (MR) of light at 820 nm for analysis of the changes in the photosynthetic activity in detached bean leaves during drying. Depending on the severity of the water deficit we identify different changes in the primary photosynthetic processes. When the relative water content (RWC) is decreased to 60% there is a parallel decrease in the ratio between the rate of excitation trapping in the Photosystem (PS) II reaction center and the rate of reoxidation of reduced PSII acceptors. A further decrease of RWC to 20% suppresses the electron transfer from the reduced plastoquinone pool to the PSI reaction center. At RWC below values 15%, the reoxidation of the photoreduced primary quinone acceptor of PSII, Q_A–, is inhibited and at less than 5%, the primary photochemical reactions in PSI and II are inactivated. Using the collected sets of PF, DF and MR signals, we construct and train an artificial neural network, capable of recognizing the RWC in a series of "unknown" samples with a correlation between calculated and gravimetrically determined RWC values of about $R2 \approx 0.98$. Our results demonstrate that this is a reliable method for determination of RWC in detached leaves and after further development it could be used for quantifying of drought stress of crop plants *in situ*. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: From Natural to Artificial Photosynthesis.

21. Gergana Savova, Katya Stankova, **Margarita Kuzmanova**, 2013, A comparison of the effects of 900 MHz EMF and gamma-ionizing radiation on human peripheral blood lymphocytes (Савова Гергана, Катя Станкова, Маргарита Кузманова, 2013, Сравнение на ефектите на 900 MHz електромагнитно поле и гама-йонизиращо лъчение върху човешки лимфоцити от периферна кръв) *BARRP Radiation Protection Journal*, (1): 46 – 50. ISSN 1314-9199.

Abstract

The usage of mobile phones increased significantly in the last 15 years. The concerns about the potential negative health effects arise, because of the daily use of electromagnetic field (EMF) sources. EMF, produced by cell phones may affect biological systems by increasing the production of free radicals, and even DNA damage. Other environmental factor, with an impact on humans' life is the ionizing radiation. The main purpose of this work is to compare the effects of 900-MHz radiofrequency fields and gamma-ionizing radiation (γ-IR) on the levels of free radicals and DNA damage in human peripheral blood mononuclear cells (PBMC). The EMF generated, at a power of 2W used for cell phone applications, led to a significant increase in the levels of intracellular reactive oxygen species (ROS), but not in persisting DNA damage 2h post-exposure. In contrast, irradiation with 4 Gy of gamma rays increased dramatically both – the intracellular ROS and the DNA damage compared to the control.

22. Kalaji H.M., A. Oukarroum, Vl. Alexandrov, **M. Kouzmanova**, M. Brestic, M. Zivcak, I.A. Samborska, M.D. Cetner, S.I. Allakhverdiev, V. Goltsev, Identification of nutrient deficiency in maize and tomato plants by *in vivo* chlorophyll *a* fluorescence measurements, *Plant Physiology and Biochemistry* 81 (2014) 16-25. doi: 10.1016/j.plaphy.2014.03.029

Abstract

The impact of some macro (Ca, S, Mg, K, N, P) and micro (Fe) nutrients deficiency on the functioning of the photosynthetic machinery in tomato (*Solanum lycopersicum* L.) and maize (*Zea mays* L.) plants grown in hydroponic cultures were investigated. Plants grown on a complete nutrient solution (control) were compared with those grown in a medium, which lacked one of macro- or microelements. The physiological state of the photosynthetic machinery *in vivo* was analysed after 14-days of deficient condition by the parameters of JIP-test based on fast chlorophyll *a* fluorescence records. In most of the nutrient-deficient samples, the decrease of photochemical efficiency, increase in non-photochemical dissipation and decrease of the number of active photosystem II (PSII) reaction centres were observed. However, lack of individual nutrients also had nutrient-specific effects on the photochemical processes. In Mg and Ca-deficient plants, the most severe decrease in electron donation by oxygen evolving complex (OEC) was indicated. Sulphur deficiency caused limitation of electron transport beyond PSI, probably due to decrease in the PSI content or activity of PSI electron acceptors; in contrary, Ca deficiency had an opposite effect, where the PSII activity was affected much more than PSI. Despite the fact that clear differences in nutrient deficiency responses between tomato and maize plants were observed, our results indicate that some of presented fluorescence parameters could be used as fluorescence phenotype markers. The principal component analysis of selected JIP-test parameters was presented as a possible species-specific approach to identify/predict the nutrient deficiency using the fast chlorophyll fluorescence records.

23. Vladimir Aleksandrov, Vasilena Krasteva, Momchil Paunov, Maria Chepisheva, **Margarita Kousmanova**, Hazem M. Kalaji and Vasilij Goltsev (2014) Deficiency of some nutrient elements in bean and maize plants analyzed by luminescent method. *Bulgarian Journal of Agricultural Science*, 20 (Supplement 1) 2014, 24–30.

Abstract

A deficiency of any essential macro (N, P, S, Ca, Mg, K) and micro (Zn, Cu, B, Mo, Cl, Mn and Fe) elements has a major influence on the development of plants. Deficits of some elements result in external features in plants. These physical marks often overlap with each other or are similar to those obtained as a result of an infection, and it is why they cannot serve as a sign for accurate diagnosis. The determination of lacking elements requires analysis of the soil or plant tissue content, or a combined analysis. Plants react to shortage of the nutrient components and, therefore, their functional analysis is preferable to soil analysis for monitoring of the nutrient deficiency. In this study the mineral deficiency in nutrient solution was evaluated by the stress response of the plants estimated by leaves photosynthetic activity. Bean (*Phaseolus vulgaris*) and maize (*Zea mays*) plants were grown hydroponically in the Hoagland nutrient medium – full or lacking K, Ca or Fe. All plants were grown in a full Hoagland nutrient solution for 10 days, and then the experimental plants were transferred to modified solution. The bean plants were decapitated after 7 days of growth in the unmodified medium. The photosynthetic activity was estimated by analysis of the chlorophyll fluorescence using JIP-test approach that reflects functional activity of Photosystems I and II and of electron transfer chain between them, as well as the physiological state of the photosynthetic apparatus as whole. The comparison of *Phaseolus vulgaris* and *Zea mays* showed different impact of each deficiency on the photosynthetic machinery of the two species. The high sensitivity of plants and specificity of primary stress reactions of the photosynthesis to mineral deficiencies outline good perspectives for fluorescent analysis application in agricultural industry. This approach is fast and cheap, and can be implemented *in vivo* and *in situ* measuring conditions.

24. Гольцев В.Н., Х.М. Каладжи, **М.А. Кузманова**, С.И. Аллахвердиев, 2014, Переменная и замедленная флуоресценция хлорофилла *a* – теоретические основы и практическое приложение в исследовании растений, ИКИ – Ижевск – Москва, 220 с., ISBN 978-5-4344-0180-7.

Goltsev V. N., Kalaji M. H., **Kouzmanova M. A.**, Allakhverdiev S. I., Variable and Delayed Chlorophyll *a* Fluorescence – Basics and Application in Plant Sciences. – Moscow–Izshevsk: Institute of Computer Sciences, 2014. 220 p.

Аннотация

В последнее время в различных странах производится большое количество разнообразных приборов для анализа состояния растений в норме и при неблагоприятном воздействии окружающей среды. Часть аппаратуры и экспериментальных методов основана на регистрации и анализе характеристик излучаемой растениями флуоресценции хлорофилла *a*. Данная книга предназначена для пользователей, не являющихся специалистами в области биофизики фотосинтеза и использующих подобные люминесцентные методы в своей практике. В книге в доступной форме рассмотрен механизм излучения флуоресценции молекулами хлорофилла антенных комплексов фотосистемы 2, описаны способы получения информации о важных характеристиках фотосинтетического аппарата. Особое внимание уделяется механизму генерации в растениях замедленной флуоресценции и дополнительным возможностям, получаемым при одновременной регистрации обоих видов флуоресценции, комбинированных с анализом сигнала рассеивания света длиной волны 820 нм. Приводится много примеров использования флуоресценции хлорофилла в изучении стрессовой реакции растений, дана информация о различных типах современных флуориметров, доступных на мировом рынке.
Книга предназначена для специалистов по экологии, физиологии, агробиологии, защиты растений, биофизики, а также для студентов, аспирантов и преподавателей биологических и аграрных вузов.

In the past few years, there have been a large number of instruments produced worldwide for the analysis of plants under normal and adverse environmental conditions. Some of the devices and their experimental approaches are based on recording and analysis of chlorophyll a fluorescence emissions from plants.
This book is intended for users who are not specialists in the field of the biophysics of photosynthesis, but are using similar luminescent methods in their activities. In the book the mechanisms of fluorescence emission by chlorophyll molecules of Photosystem II antenna complexes are described in an understandable way and useful algorithms are provided for extraction of the necessary information about important characteristics of the photosynthetic machinery.
Special attention is focused on the mechanisms of delayed fluorescence generation in plant tissues. The simultaneous recording of both types of fluorescence (prompt and delayed) combined with the analysis of the light reflection signal at 820 nm wavelength provides additional opportunities for plant research. Numerous examples of chlorophyll fluorescence applications for monitoring of plant stress responses also provide information about the various types of modern fluorometers available throughout the world. The book is recommended for specialists in the fields of ecology, physiology, agrobiology, plant protection and biophysics and also as a useful reference for students of MS and Doctorate Degrees.

25. Ekaterina K. Yotsova, Anelia G. Dobrikova, Martin A. Stefanov, **Margarita Kouzmanova**, Emilia L. Apostolova. 2018. Improvement of the rice photosynthetic apparatus defence under cadmium stress modulated by salicylic acid supply to roots. *Theor. Exp. Plant Physiol.* 30:57–70. https://doi.org/10.1007/s40626-018-0102-9

Abstract

The present study was conducted to investigate the effect of exogenous salicylic acid (SA) added to the nutrient solution on the growth parameters and the functions of the photosynthetic apparatus of rice plants under cadmium (Cd) stress. Our investigations have shown that 10 μM SA has an optimal effect in rice plants grown hydroponically. Pulse amplitude modulated chlorophyll fluorescence, low-temperature chlorophyll fluorescence, oxygen evolution (measured with Clark-type and Joliot-type electrodes) and P700 photo-oxidation measurements were carried out to assess the effect of SA on the activity of the photosynthetic apparatus. The levels of three important parameters associated with oxidative stress (hydrogen peroxide, lipid peroxidation and proline content) were measured. The application of low concentration of SA significantly decreased the levels of hydrogen peroxide, lipid peroxidation and proline under Cd stress. The results revealed that low concentration of SA, applied in plants exposed to 150 μM $CdCl_2$, significantly improves plant growth, photochemical activities of both photosystems, the electron flow from Q_A to plastoquinone, energetic distribution between pigment-protein complexes and the kinetic parameters of oxygen-evolving reactions. This study suggests that exogenous application of 10 μM SA through the rooting medium has a protective effect against Cd toxicity in rice plants. The possible molecular mechanisms involved in the defense effect of SA on the function of photosynthetic apparatus are discussed.

26. Izabela A. Samborska, Hazem M. Kalaji, Leszek Sieczko, Wojciech Borucki, Radosław Mazur, **Margarita Kouzmanova**, Vasilij Goltsev. Can just one-second measurement of chlorophyll a fluorescence be used to predict sulphur deficiency in radish (*Raphanus sativus* L. sativus) plants?, *Current Plant Biology*, Available online 19 December 2018, Volume 19, September 2019, 100096, open access https://doi.org/10.1016/j.cpb.2018.12.002

Abstract

Nutrients deficiency (ND) in plants is one of the main factors adversely affecting plant growth and yield of agricultural and horticultural production. Usually detection of NDs in plants is based on visible symptoms or destructive chemical analysis. Recently chlorophyll a fluorescence is one of the most used approaches to investigate photosynthesis light-dependent reactions. It is widely accepted that it can inform about structural and functional changes in photosynthetic apparatus, especially under abiotic stresses including NDs. The aim of this research was to study the influence of sulphur deficiency on photosynthetic efficiency of two cultivars radish (*Raphanus sativus* L. sativus) – 'Fluo HF1' and 'Suntella F1', and to assess the potential of chlorophyll a fluorescence measurements in early detection of this stress. Our results showed the sulphur deficiency has decreased the electron transport rate, chlorophyll content, grana size, and, at some degree, net photosynthesis and stomatal conductance. The changes in photosynthetic machinery performance were observed earlier than the visible effects of sulphur deficiency. However, the photosynthetic apparatus of radish plants was not considerably affected by this stress. Some sensitive parameters of OJIP test were altered but we were not able to identify the sulphur deficiency stress based on the alteration of only one of these parameters. We conclude that the detection of specific stress reactions of plants to sulphur deficiency should be based on integrative analysis of the response of the photosynthetic machinery. Such stress induced reactions can be detected by Principal Component Analysis of a large number of JIP-test parameters.

27. Najafpour, Mohammad; Zaharieva, Ivelina; Zand, Zahra; Hosseini, Seyedeh; **Kouzmanova, Margarita;** Hołyńska, Małgorzata; Tranca, Ionut; Larkum, Anthony; Shen, Jian-Ren; Allakhverdiev, Suleyman. (2020). Water-oxidizing complex in Photosystem II: Its structure and relation to manganese-oxide based catalysts. *Coordination Chemistry Reviews*. 409. DOI: 10.1016/j.ccr.2020.213183.

Abstract

Cyanobacteria, green algae, and higher plants provide the major part of molecular O_2 of Earth atmosphere via water oxidation of oxygenic photosynthesis. The water-oxidizing complex is a manganese-calcium oxide-based cluster embedded in Photosystem II that oxidizes water with high turnover frequency. The atomic structure and analysis of the Mn-Ca cluster are important in understanding the mechanism of water oxidation and for the design of efficient artificial water-oxidizing catalysts. With this short review, we aim to introduce the basic features of the biological water oxidation to the new-comers in the field. Taking into account the recent structural studies, including a high-resolution, radiationdamage-free structure of the water-oxidizing complex, and structures of intermediate S-states revealed by femtosecond X-ray free electron lasers, we discuss the structure and functions of the biologically active site and its implications for the development of inorganic catalysts for solar fuels production.

28. Zagorchev L., Traianova A., Teofanova D., Li J., **Kouzmanova M.**, Goltsev V. (2020). Special issue in honour of Prof. Reto J. Strasser – Influence of *Cuscuta campestris* Yunck. on the photosynthetic activity of *Ipomoea tricolor* Cav. – *in vivo* chlorophyll *a* fluorescence assessment. *Photosynthetica*. 58 (SI): 237–247. DOI:10.32615/ps.2020.004.

Abstract

Cuscuta campestris Yunck. is a parasitic plant, acquiring nutrients from the hosts. Although a generalist, its hosts differ in their susceptibility. *Ipomoea tricolor* Cav. is a semi-compatible host – *C. campestris* growth is restricted in infected plants. We aimed to assess the effect of the parasite on this semi-compatible host by using the sensitive JIP-test to follow physiological changes in leaves at different vegetative stages – aged, mature, and newly emerging. The characteristics of the photosynthetic machinery were estimated by 17 parameters, calculated from the prompt chlorophyll *a* fluorescence. The most sensitive were performance index of photosystem II, performance index of photosystems I + II, and number of QA redox turnovers until maximal fluorescence is reached. The infected *I. tricolor* plants responded to the parasite by activating the electron transport in PSII in later periods. The oldest leaves and the youngest leaves developed certain adaptation to the parasite but the younger did not. The effect of the parasite on the photosynthetic apparatus depended on the physiological age of the host plant leaves.

29. Plyusnina T.Yu., Khruschev S.S., Degtereva N.S., Konyukhov I.V., Solovchenko Al., **Kouzmanova M.**, Goltsev V.N., Riznichenko G.Yu., Rubin A.B. (2020). Special issue in honour of Prof. Reto J. Strasser – Gradual changes in the photosynthetic apparatus triggered by nitrogen depletion during microalgae cultivation in photobioreactor. *Photosynthetica*. 58 (SI): 443-451, 2020. DOI: 10.32615/ps.2020.002.

Abstract

Changes of photosynthetic activity during natural depletion of nitrogen in a batch culture of *Chlorella vulgaris* were studied using chlorophyll *a* fluorescence-based methods. Complex analysis including JIP-test, multiexponential approximation, and the analysis of differential curves was carried out on the recorded fluorescence transients. Three pivot points were detected in dynamics of the JIP-test parameters during culture growing. We associated these time points with different stages of mineral stress progress. Immediately after nitrogen exhaustion in the cultivation medium, a transient increase in the efficiency of PSII was detected. During this short period, the photosynthetic apparatus acclimated to the stress by reducing the number of PSII reaction centers, simultaneously increasing the effective cross section of the light-harvesting antenna per reaction center. However, prolonged nutrient starvation impaired the structure of the photosynthetic apparatus, deactivating the oxygen-evolving complex and impairing the overall electron transport.

30. Dimitrova S., Paunov M., Pavlova B., Dankov K., **Kouzmanova M.**, Velikova V., Tsonev T., Kalaji H., Goltsev V. (2020). Special issue in honour of Prof. Reto J. Strasser – Photosynthetic efficiency of two *Platanus orientalis* L. ecotypes exposed to moderately high temperature – JIP-test analysis. *Photosynthetica*. DOI: 10.32615/ps.2020.012.

Abstract

Plane (*Platanus orientalis*) is a popular park tree in Europe but is almost extinct in natural ecosystems, because of climate changes. In our study, two ecotypes of plane tree (Bulgarian and Italian) were submitted to moderately elevated temperature that occurs in summer. Our aim was to compare stress reactions, tolerance, and adaptability of these plants. Leaf age had a significant impact on the stress effects. For correct interpretation of the results, we investigated leaves at different positions from the apical bud, i.e., leaves of different age. We assessed their photosynthetic efficiency at room temperature and after treatment at moderately high temperature by simultaneous measurement of prompt and delayed chlorophyll fluorescence, as well as light reflection at 820 nm. For more precise interpretation of the obtained results we did principal component analysis. The two studied plane ecotypes showed different tolerance to the elevated temperature. Plants of Italian ecotype showed better adaptivity and developed advantageous photosynthetic characteristics, while Bulgarian ecotype was more affected. Plants of both ecotypes recovered from the heat stress.

31. Zagorchev, L.; Atanasova, A.; Albanova, I.; Traianova, A.; Mladenov, P.; **Kouzmanova, M.**; Goltsev, V.; Kalaji, H.M.; Teofanova, D. Functional Characterization of the Photosynthetic Machinery in *Smicronix* Galls on the Parasitic Plant *Cuscuta campestris* by JIP-Test. *Cells* 2021, *10*, 1399. https://doi.org/10.3390/cells10061399

Abstract

Members of the genus *Cuscuta* are generally considered to be non-photosynthetic, stem-holoparasitic flowering plants. Under certain circumstances, at least some members of the genus are capable of limited photosynthesis. The galls of the *Smicronyx* weevils formed on *Cuscuta campestris* are particularly rich in chlorophylls compared to the stem of the parasitic plant. In the present study, we aimed to characterize the photosynthetic activity in the inner and outer gall cortices in comparison to the non-photosynthetic stems and a reference plant (*Arabidopsis thaliana*). The recorded prompt chlorophyll fluorescence transients were analyzed using JIP test. Detailed analysis of the chlorophyll fluorescence confirmed the presence of actively functioning photosynthetic machinery, especially in the inner cortex of the galls. This photosynthesis, induced by the insect larvae, did not reach the levels of the photosynthetic activity in *Arabidopsis thaliana* plants. Thylakoid protein complexes were identified by separation with two-dimensional Blue Native/SDS PAGE. It appeared that some of the complexes presented in *A. thaliana* are missing in *C. campestris*. We hypothesize that the insect-triggered transition from non-photosynthetic to photosynthetic tissue in the gall is driven by the increased requirements for nutrients related to the larval nutrition.