

UDC 581.17

Biophysics

IMPACT OF STRESS FACTORS ON THE GENERATION OF NANOPARTICLES IN THE BIOLOGICAL STRUCTURES

A.N.Nasibova¹, B.V.Trubitsin², S.M.Ismayilova³, I.Y.Fridunbayov³,
U.M.Gasimov¹, R.I.Khalilov³

(Presented by Academician of ANAS F.A.Kadirov)

This article has been devoted to the research of the formation of iron oxide nanoparticles in some of the plant and soil samples and the impact of stress factors in this event. Studied element analysis of leaves plants, which widespread in the territory of Lankaran - lemon, orange, magnolia, kiwi and feijoa. Taken EPR spectra of these plant samples and taken from they are grown soil samples. This specters prove that the magnetic nanoparticles is formed in plants and at the same time they some extent accumulation in plants from the soil. In addition in this article was studied the formation of magnetic nanoparticles in chloroplasts, as well as wheat and maize plantlets and impact of various factors (various concentrations of FeCl₂, FeCl₃ and of NaCl) to the formation of nanoparticles. Has been known, that a certain dose of stress factor that stimulates the formation of nanoparticles in plants.

Keywords: plants, chloroplast, nanoparticles, stress factor, paramagnetic centers, EPR signals, element analysis

Nanotechnology is a field of fundamental, at the same time applied science and technology. There is no area of human activity, where should not be applied the nanotechnology. The rapid development of nanotechnology is resulted to the production of new nanomaterials, nanostructures and nanoparticles applied in many areas. Currently, needed to the use of nanoparticles in different fields is synthesized by different methods. It is known that the synthesis of nanoparticles in biological way is very favorable of ecological safe and economically. Therefore, biosynthesis of various nanoparticles now is one of the areas interested world scientists [5,6].

In addition to the biosynthesis of nanoparticles, the study of their generation in biological systems are of great interest. Our studies was dedicated to the study of the generation of different nanoparticles in living systems, to clarify the molecular mechanism of this event, to the implementation of the biosynthesis of different nanoparticles in living systems and to study the impact of stress factors (radioactive contamination, gamma-radiation, UV radiation, etc.) to all these events.

Materials and methods

Our research samples has been fresh leaves of wide-spread plants in the territory of Lankaran – lemon, orange, magnolia, kiwi, feijoa and soil samples collected from those territories. In these areas the exposure dose rate has been appointed with dosimeter-radiometer (MKC-AT 1125, Atomtex, Belarus). Samples were pulverized after drying at room temperature and with EPR (Electron Paramagnetic Resonance) spectrometer were taken their spectra (Varian E-4-US, Bruker - Germany). Element composition of samples have been identified through XRF-Analyzer, Innov-X (USA) X-ray fluorescence spectrometry. In experiments conducted with wheat and maize seeds they are germinate in water added of different concentrations (0.05 M, 0.1M, 0.2 M) NaCl. Then dried at room temperature and has been into powder. EPR spectra of the prepared samples were considered. The next experiments carried out by chloroplasts separated from higher plants (spinach). In experiments were prepared the chloroplast solutions. To the 50 mkl chloroplast solution have been added FeCl₂, FeCl₃ (10⁻²M, 10⁻³M, 10⁻⁴ M, 10⁻⁵ M) and metilviologen (as electron acceptor).

Result and discussions

Above-mentioned plants and soil samples when collecting from the territory of Lankaran, radiation background has been designated by the dosimeter-radiometer. In these areas, radiation background has changed in the range of 2-4 mkR/h and this corresponds to the normal natural radiation background for our Republic. Were registered EPR spectra of the collected samples after drying at room temperature in the summer months (fig.1). Note that the first time formation of iron oxide magnetic nanoparticles in plants is shown by the EPR method by us. According to parameters we can say, that received signal (fig.1A) belonged to the magnetic nanoparticles. At the same time we have observed EPR signals of paramagnetic centers that characterizes of iron components in the collected soil samples in the field, where plants grown (fig.1B).

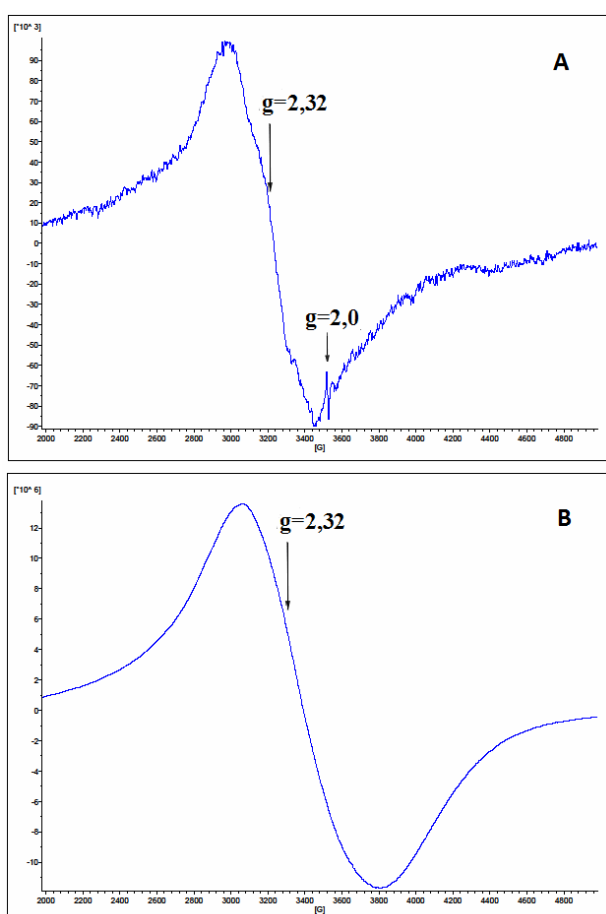


Fig.1. The EPR signals of the dried leaves of lemon (A) and soil samples taken at this territory (B)

In fig.1 given EPR signals of dried leaves of lemon (A) and soil samples taken at this territory (B). Parameters of received EPR signals ($g = 2.32$, $\Delta H = 500$ Gs) from lemon leaves shows the existence of iron oxide magnetic nanoparticles in this leaves. In addition by us was examined the EPR signals of the dried leaves plants collected in Lenkoran - tangerine, king kong, magnolia and feijoa. Receiving wide EPR signals characterizes magnetic nanoparticles also on these plants is of great interest. To study the properties of special wide EPR signals characterizing iron oxide nanoparticles we have researched EPR signals of magnolia by changing parameters of radiospectrometer. So to determine the effect of the angle in the resonator, when researching in different angles (90° , 180°) glass ampoule with samples, we have observed a change the form of EPR signal (fig.2). It shows that paramagnetic centers responsible for EPR signal have a complex structure. In future researches continued clarification of this "angle effect".

Has been appointed element composition of plant samples collected from Lankaran and soil samples taken from this area (table 1). As a result of element analysis of soil and plants each one of them were found in large quantities K, Ca, Mn, Fe and Sr.

In leaf samples K and Ca, but in soil samples Mn and Fe have been identified most of all. Elements Ti and Ba has not been in plant samples, but in soil samples they were found sufficiently.

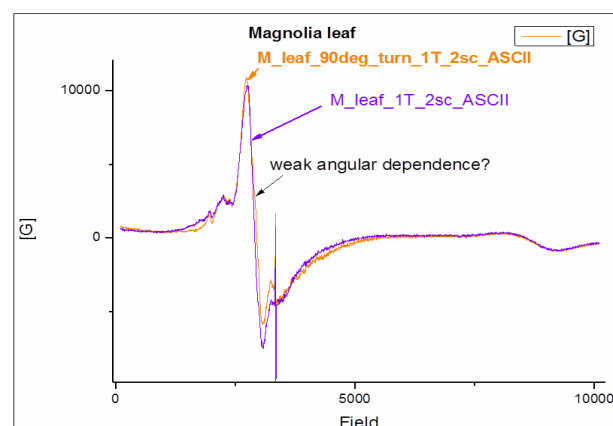


Fig.2. The EPR signals of the dried magnolia leaves

Table 1

Sample title	vahid	S	Cl	K	Ca	Ti	Cr	Mn	Fe
Limon - leaf	ppm	35070	<LOD	102672	259999	<LOD	<LOD	247	1508
Orange - leaf	ppm	21269	21363	63102	186844	<LOD	58	163	1901
Magnolia - leaf	ppm	41502	<LOD	37558	77087	<LOD	80	588	852
Feijoa - leaf	ppm	<LOD	5565	76709	24189	<LOD	89	229	913
Kiwi - leaf	ppm	68829	11132	108096	137689	<LOD	<LOD	348	1807
Magnolia - soil	ppm	<LOD	<LOD	26306	29523	5839	165	863	47659
Lemon - the soil	ppm	<LOD	<LOD	23042	28890	6589	193	738	56607
Orange - the soil	ppm	<LOD	<LOD	27429	23310	5179	109	940	47455
Kiwi - soil	ppm	<LOD	<LOD	21349	28296	4732	143	765	38774
Feijoa - the soil	ppm	<LOD	<LOD	26305	34335	5635	157	1115	51033

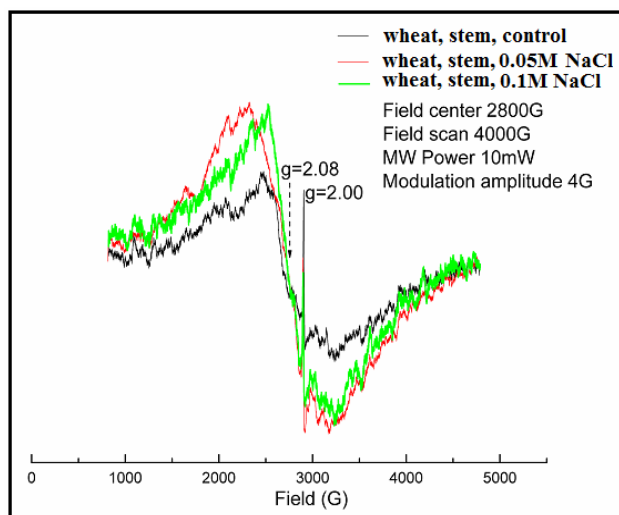
Sample title	vahid	Co	Zn	Rb	Sr	Zr	Mo	Ba
Limon - leaf	ppm	<LOD	71	20	633	60	<LOD	<LOD
Orange - leaf	ppm	<LOD	244	17	528	61	63	<LOD
Magnolia - leaf	ppm	<LOD	63	22	141	82	83	<LOD
Feijoa - leaf	ppm	<LOD	59	37	69	65	67	<LOD
Kiwi - leaf	ppm	<LOD	<LOD	<LOD	297	88	93	<LOD
Magnolia - soil	ppm	<LOD	72	67	476	101	<LOD	456
Lemon - soil	ppm	<LOD	52	63	523	87	<LOD	499
Orange - soil	ppm	373	199	79	445	139	<LOD	553
Kiwi - soil	ppm	<LOD	181	68	384	108	<LOD	361
Feijoa - soil	ppm	<LOD	95	64	584	83	<LOD	506

When we studying the formation of iron oxide magnetic nanoparticles in plants, along with plants collected from Lankaran, we have conducted experiments with wheat and maize seeds. We studied the impact of different concentrations of NaCl to the formation of magnetic nanoparticles in seedlings of this plants.

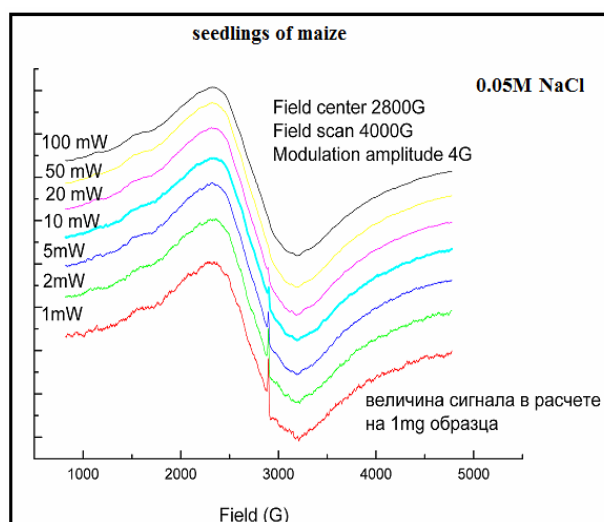
Wheat and maize seeds were grown in water into which added in different concentrations (0.05 M, 0.1M, 0.2 M) NaCl. Then seed shoots and roots dried at room temperature, was ground to a powder and registered EPR spectra of them. Influence of the NaCl in different concentrations to the EPR signals characterizes magnetic nanoparticles were analyzed by means of this spectra (fig.3).

When we comparing the EPR signals of wheat seeds (9 day) we see that amplitude of EPR signals seedlings growing in distilled water significantly smaller of the amplitude EPR signals seedlings growing in the water, into which added different concentrations (0.05 M, 0.1M) NaCl (fig.3A). This difference more clearly has been observed in signals of roots (fig.3B, C).

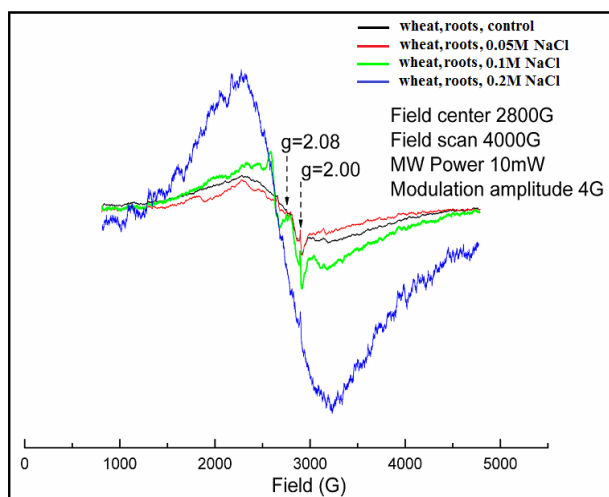
The results show that the amplitude of characteristic broad range EPR signal's of seed, as well as root samples with increasing of NaCl concentration is even higher. As received signals characterizing metal nanoparticles, we can say that stress factor has a stimulating effect on the formation of magnetic nanoparticles in plants.



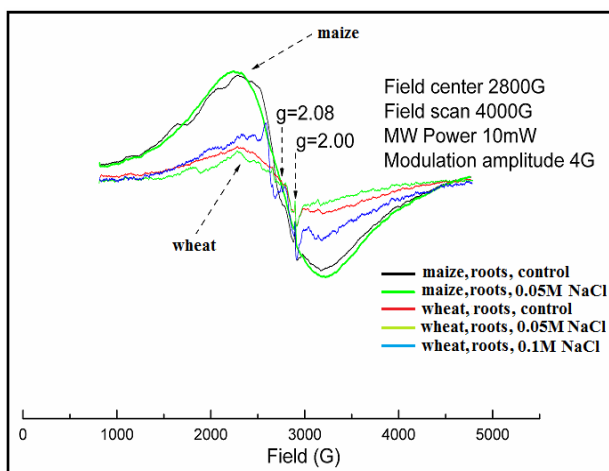
A



D



B



C

Fig.3. EPR spectra of wheat and maize seedlings

It should be noted that in previous studies we also received analogical results when studying the impact of radioactive contamination and ionizing gamma radiation on the formation of iron oxide magnetic nanoparticles in plants. We showed that radiation factor until a certain dose (lethal dose) has a stimulating effect to the formation of nanoparticles in plants [2, 3].

In fig. 3 we have identified the dependence of the behavior free radical signal and the power of electromagnetic waves. By us in addition to the *in vivo* studies have been fulfilled *in vitro* studies also. In this case, experiments were conducted with chloroplasts obtained from higher plants (spinach). After adding the salts FeCl_2 and FeCl_3 in concentrations 10^{-3} - 10^{-5}M to the chloroplasts (spinach) considered EPR spectra of them. When adding FeCl_2 the amplitude of chloroplast's EPR signal on light which characterizes magnetic nanoparticles was high than the amplitude of the signal in the dark. But with the addition of FeCl_3 the amplitude of chloroplast's EPR signal on light has been considerably smaller in comparison with signal in the dark (fig.4).

We have observed magnetic nanoparticles in chloroplasts in the initial experiments conducted by Transmission Electron Microscope (fig.5).

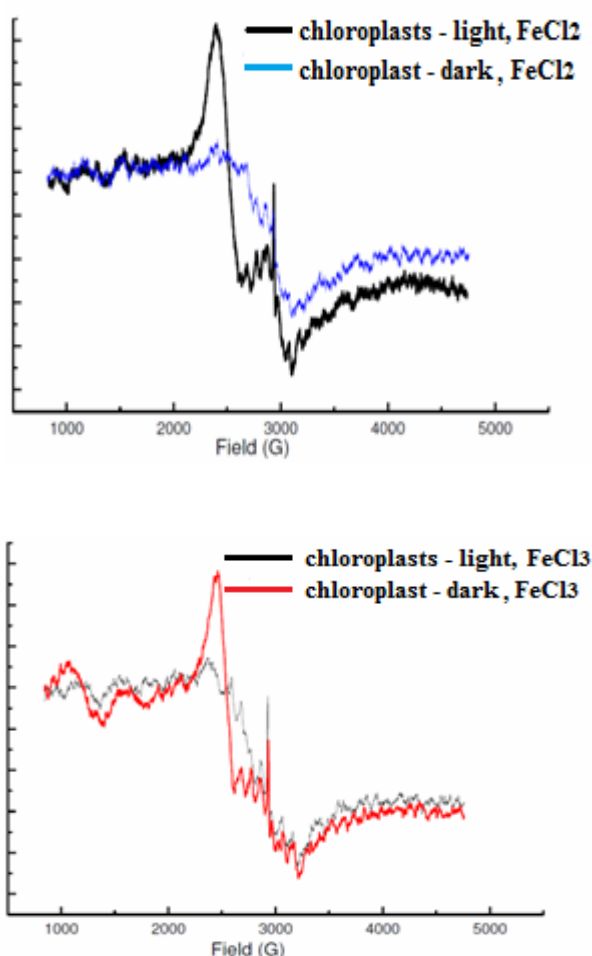


Fig.4. EPR spectra after adding to the chloroplast FeCl_2 and FeCl_3 in the dark and light.

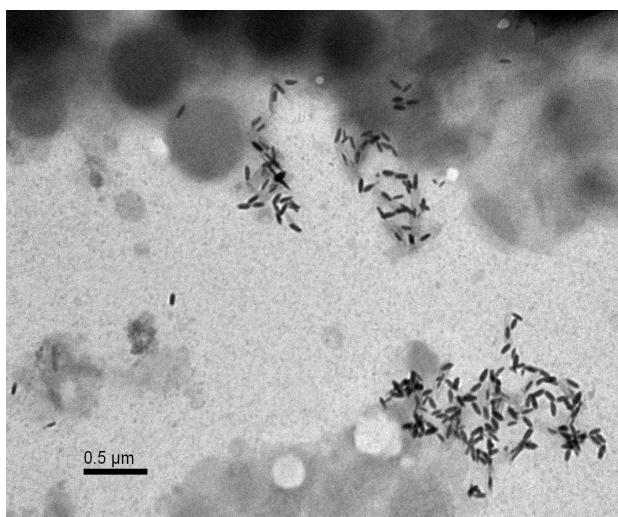


Fig.5. TEM description of chloroplast solution addition of iron salt

On the basis of conducted experiments has been identified that the formation of magnetic nanoparticles may be associated with activities photosynthetic apparatus in plants and in the process of incubation of samples depends of their lighting conditions. The results show that the formation processes of magnetic nanoparticles in plants can be connected with violation of intactness chloroplasts and other components. As a result, is provided the availability of the precursors of nanoparticles (eg., iron ions) in electron transport chain of chloroplast membranes, during of the oxidation-reduction reactions are formed magnetic nanoparticles. Any stress factor until definition dose has a stimulating effect to this event.

This work have been carried out with the assistance of the Science Development Foundation under the President of the Republic of Azerbaijan - Grant EIF-2012-2(6)-39/20/3-M-11

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¹ Institute of Radiation Problems of ANAS

² Moscow State University

³ Baku State University

BIOLOJİ STRUKTURLARDA NANOHISSƏCİKLƏRİN GENERASIYASINA STRESS AMİLLƏRİN TƏSİRİ

A.N.Nəsibova, B.V.Trubitsin, S.M.İsmaylova, İ.Y.Fridunbəyov,
Ü.M.Qasımov, R.İ.Xəlilov

Məqalə bəzi bitki və torpaq nümunələrində dəmir oksidi nanohissəciklərinin formalaşmasının və bu hadisəyə stress amillərin təsirinin tədqiqinə həsr edilmişdir. Lənkəran torpaqlarında geniş yayılmış – limon, portağal, maqnoliya, kivi və feyxoa bitkilərinin element analizi öyrənilmiş, bu bitkilərin və onların bitdiyi yerdən götürülmüş torpaq nümunələrinin EPR spektrləri çəkilməmişdir. Alınan spektrlər bitkilərdə maqnit nanohissəciklərinin əmələ gəldiyini və onların eyni zamanda torpaqdan da müəyyən qədər bitkilərə daxil olduğunu sübut edir. Bundan başqa məqalədə xloroplastlarda, eləcə də buğda və qarğıdalı cücərtilərində maqnit nanohissəciklərinin əmələ gəlməsi və bu nanohissəciklərin əmələ gəlməsinə müxtəlif amillərin (müxtəlif qatılıqlarda FeCl_2 , FeCl_3 və NaCl -un) təsiri öyrənilmişdir. Məlum olmuşdur ki, stress amil müəyyən dozada bitkilərdə nanohissəciklərin əmələ gəlməsinə stimullaşdırıcı təsir göstərir.

Açar sözlər: bitkilər, xloroplast, nanohissəciklər, stress amil, paramaqnit mərkəzlər, EPR signalları, element analizi

ВЛИЯНИЕ СТРЕСС ФАКТОРОВ НА ГЕНЕРАЦИЮ НАНОЧАСТИЦ В БИОЛОГИЧЕСКИХ СТРУКТУРАХ

А.Н.Насибова, Б.В.Трубицин, С.М.Исмаилова, И.Ю.Фридунбеков,
У.М.Касумов, Р.И.Халилов

Работа посвящена исследованию формирования наночастиц оксидов железа в образцах почвы и растений, а также влияния стресс факторов на этот процесс. Изучен элементный анализ растений, широко распространенных в Ленкаране – лимона, апельсина, магнолии, киви и фейхоа. Зарегистрированы спектры ЭПР образцов растений и почвы, в которых растут эти растения. Полученные спектры доказывают образование магнитных наночастиц в растениях и одновременно их накопление из почвы в небольшом количестве. Изучено формирование магнитных наночастиц в хлоропластах, а также в проростках кукурузы и пшеницы, и влияние различных факторов (FeCl_2 , FeCl_3 , NaCl в разных концентрациях) на образование этих наночастиц. Выяснено, что стресс фактор оказывает стимулирующее действие на формирование наночастиц в растениях.

Ключевые слова: растения, хлоропласты, наночастицы, стресс фактор, парамагнитные центры, сигналы ЭПР, элементный анализ