BIOCOMPATIBLE MAGNETIC FLUID NANOPARTICLES INTERNALIZED IN VEGETAL TISSUE

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Received December 15, 2007

This experimental study was focused on the study of assimilatory pigments and nucleic acid levels in young plants intended for agricultural use (Zea mays) in presence of water based magnetic fluid in culture medium. The water based magnetic fluid was constituted by coating the small magnetic nanoparticles with perchloric acid and further dispersion in water. Saturation magnetization of our magnetic fluid was of 18.3kA/m; the magnetic nanoparticles volume fraction was of 4.5% while the average particle diameter was equal to 10.55nm. After germination, daily supply with 10mL magnetic fluid aqueous suspension per dish of sample was carried out for 12 days, plant growth being conducted in controlled conditions into a laboratory room. Water based magnetic fluid was added daily in various volume fractions in deionized water (50–100–150–200 and 300µl/l) in the culture medium of Zea mays plantlets in their early ontogenetic stages. Volume fraction of aqueous magnetic fluid solution added in culture medium had a slight inhibitory effect on the growth of the plantlets. We have noticed that toxicity symptoms led to brown spots covering the leaf surface for the enhanced volume fraction of aqueous magnetic fluid solution added by us in the plants culture medium in this experiment. The iron excess treatment is believed to generate oxidative stress in leaf cells. In this case, photosynthesis might be affected leading to decrease of the metabolic process rate.

Key words: magnetic nanoparticles, Zea mays plants growth, photo-assimilatory pigments, nucleic acids.

1. INTRODUCTION

The biological interest in the magnetic fluid effect in living organisms represents an important application field of magnetic nanoparticles, mainly for


biotechnological use. Stable concentrated suspensions of magnetic nanoparticles in either organic or inorganic solvents are called magnetic fluids [1]. Magnetic fluids are suspensions of superparamagnetic particles – small single domain particles – that respond to a magnetic field but completely demagnetize when the field is removed. Colloidal stability is assured by coating the particle with a shell of nonmagnetic molecular surfactants which prevent close approach of the nanomagnetic cores thereby reducing the possibility of aggregation via Van der Waals or magnetic attractions. For medical purposes the ferrophase need also to have small physical diameter in order to pass through the cell biomembranes and to reach various target tissues.

In the last time the interest for the study of the biological effects induced by magnetic fluid presence in culture medium upon vegetal organisms [2, 3] and microorganisms [4] as well as upon animals [5] has increased. Special attention was paid to genetic effects of magnetic fluid nanoparticles that are found to lead to chromosomal aberrations in young vegetal plants [6–7] which may be related to the putative use in plant biotechnology. Relatively small number of experimental studies is dedicated to the influence of magnetic fluids on the photosynthesis process [2, 8, 9] revealing the stimulatory effect of magnetic fluids on the chlorophylls content, which has evidenced some stimulatory effects on the plant growth. This stimulatory effect may be explained on the basis of iron importance in the vegetal organisms [10–11].

Lobreaux et al. [12] have reported that iron treatment of Zea mays induced ferritin protein accumulation in roots and leaves over a period of 3 days. Recently investigations have shown that phytoferritin occurs in plant cells as crystalline magnetite (Fe₃O₄), maghemite (α-Fe₂O₃), and hematite (γ-Fe₂O₃) [13]. The biological interest in the magnetic fluid effect in living organisms represents an important application field of magnetic nanoparticles, mainly for biotechnological use.

In this paper the authors present some quantitative observations regarding the influence of magnetic nanoparticles coated with perchloric acid on the growth of Zea mays plants in early ontogenetic stages.

2. MATERIALS AND METHODS

The present experimental study was focused on the assimilatory pigments and nucleic acid levels in young plants intended for agricultural use (Zea mays) in presence of water based magnetic fluid in culture medium. The water based magnetic fluid was constituted by coating the small magnetic nanoparticles with perchloric acid and further dispersion in water. Saturation magnetization of our magnetic fluid was of 18.3kA/m; the magnetic nanoparticles volume fraction was of 4.5% while the average particle diameter was equal to 10.55nm. The ferrophase content was of 2.03·10¹⁷ particles within 1ml of the initial magnetic fluid.
Seeds from a single plant (in order to diminish the putative genophond variations) were let to germinate on watered porous paper support in Petri dishes (each sample was compound of 50 seeds) in darkness and suitable temperature. After germination, daily supply with 10ml magnetic fluid aqueous suspension (vigorously shaking) per dish of sample was carried out for 12 days, plant growth being conducted in controlled conditions of temperature (22.0±0.5°C), illumination (dark/light cycle: 14h/10h) and 90% humidity into a laboratory room. Water based magnetic fluid was added daily in various volume fractions in deionized water (50–100–150–200 and 300µl/l) in the culture medium of Zea mays plantlets in their early ontogenetic stages.

After 12 days of plant growth the Lichtenthaler & Welburn’s method [14] was to assay the chlorophyll a, chlorophyll b and total carotenoid pigments while a modified Spirin’s method [15, 16] for nucleic acids assay was used.

Biological material consisted of green tissue obtain by mixing up the green tissue from the all young plantlets grown from each experimental group. The spectral device was a CINTRA 5 spectrophotometer UV-VIS provided with quartz cells. Three repetitions of experimental investigations about assimilatory pigments and nucleic acids extraction and spectrophotometric assays were carried out for all experimental variant samples. Average values, standard deviations and t-test have been considered for statistical analysis. Plant individual length was measured with 0.1cm precision and statistically analysis was accomplished by means average plant lengths, standard deviation and confidence interval, calculated for each batch of plantlets using the Student t-test.

2. RESULTS AND DISCUSSIONS

We have noticed that toxicity symptoms led to brown spots covering the leaf surface for the enhanced volume fraction of aqueous magnetic fluid solution added in the plants culture medium in this experimental investigation (Fig. 1).

![Fig. 1. –Toxicity symptoms on leaf surface of 12 days old Zea mays plantlets.](image-url)
The iron excess treatment generated oxidative stress in leaf cells and thus the photosynthesis processes may be greatly affected leading to decrease of the process rate. The lengths of the 12 days plantlets were carefully measured with 0.1 cm precision. The average lengths and the standard deviations were calculated for each batch of test seeds. The confidence interval was calculated for every batch of plantlets using the Student test, for the confidence level \( P = 95\% \). Fig. 2 presents the average plants length for each volume fraction of magnetic fluid solution supplied to the test samples. We found that the enhanced volume fractions of the magnetic fluid solution have an inhibitory effect on the plants growth.

![Average length, P=95%](chart.png)

**Fig. 2.** – The average length versus volume fraction of magnetic fluid solution added in culture medium.

The results are statistically significant with one exception in the 50\(\mu l/l\) sample, as resulted from the average comparison with the lengths of the control, using the Student t-test. Seem to be that the iron excess in our samples, provided by magnetic fluid nanoparticles, induced the slow down of plants growth.

The contents of photosynthesis pigments (\(a\) and \(b\) chlorophylls and total carotenoids) in the green tissue of young *Zea mays* plantlets (aged of 12 days) for experimental samples in Fig. 3 are presented.

The chlorophyll \(a\) level, the main photosynthesis pigment, was found easy decreased for increased volume fraction of magnetic fluid solution added in culture medium, thus a slow inhibitory effect was noticed. Similar response was get for the other all photo-assimilatory pigments analyzed.

Linear correlation was evidenced between chlorophyll \(a\) (Chl \(a\)) and chlorophyll \(b\) (Chl \(b\)) levels (Fig. 4), the correlation coefficient, \(R^2\), being over
0.95. As known, the role of carotenoid pigments is to sustain the photosynthesis process by transferring the energy absorbed from the environmental light to the molecules of chlorophyll a – that are able to catalyze the electromagnetic energy conversion into its chemical form (this resulting in the biosynthesis of saccharides, proteins and lipids). The total assimilatory pigments contents have the same variation to the increased values of volume fraction of magnetic fluid solution added in the culture medium of young plantlets that was observed for all pigments level.

Fig. 3. – Assimilatory pigments level in *Zea mays* plantlets *versus* volume fraction of magnetic fluid solutions (Chl a –the content of chlorophyll a, Chl b-the content of chlorophyll b, Car-the content of total carotenoid pigments).

Fig. 4. – Linear correlation between chlorophyll a (Chl a) and chlorophyll b (Chl b) pigments level in *Zea mays* plantlets.
The best indicator upon the photosynthesis process efficiency is considered the chlorophylls ratio (chlorophyll a / chlorophyll b) [17] which provides indirect information on the enzymatic aggregates of the Light Harvesting Complex II (LHC II) from the photosynthetic system II located in the chloroplasts membranes.

In Fig. 5 a slow stimulatory influence of increased volume fractions of magnetic fluid solution added in culture medium of plants, to photosynthesis process, as suggested by chlorophyll a and b ratio, can be seen for the relative low volume fractions of magnetic fluid solutions; an inhibitory effect is obvious for the highest volume fraction of magnetic fluid solution. This can be taken as a conclusive proof of the capacity of the water-magnetic fluid to influence the LHCII enzyme system.

![Graph](image.png)

**Fig. 5.** – The effects of different volume fraction of magnetic fluid solutions added in culture medium on chlorophylls ratio.

The statistical analysis accomplished for the chlorophyll ratio (by applying the t-test to compare control and test sample data) revealed statistic significance (p<0.05) for all samples under magnetic fluid influence except for the sample supplied with 150µl/l volume fraction of magnetic fluid solution.

The total content of nucleic acids (DNA+RNA) in young *Zea mays* plantlets after 12 days of grown under different volume fraction of magnetic fluid solutions added in plants culture medium is presented in Fig. 6.

One can see that for increasing volume fraction of magnetic fluid solution the average nucleic acid level is decreased in comparison to the control sample,
revealed an inhibitory effect on biosynthesis. Applying the t-test to compare control and test sample, data for the average nucleic acid level statistic significance (p<0.05) was found for all samples under magnetic fluid influence.

![Nucleic acids levels in Zea mays plantlets grown under magnetic fluid influence.](image)

We suppose that the iron oxides provided by the magnetite from magnetic fluid ferrophase could interfere with the complex redox reactions involved in the photosynthesis phenomenon. Another supposition about magnetic fluid influence on the photosynthesis process is the iron uptake in the form of iron chelates, known as phyto-siderophores, discussed since the putative siderophore presence in the thylakoidal membranes could result in some changes during the biochemical reactions from the vegetal cells. The iron excess treatment is generated oxidative stress in leaf cells.

The major form of iron available to plants in well aerated soils is the relatively insoluble ferric form (Fe\(\text{III}\)) [18]. However there is the reduced ferrous (Fe\(\text{II}\)) form which is actually absorbed by roots [19]. It is assumed that siderophores biosynthesis – evidenced mainly in bacteria cells [20], can be an important process of ferric iron reducing. Among various bacteria species able of iron chelation in the form of siderophores there are certain *Pseudomonas aeruginosa* strains (this species being known for its numerous ecological nishes, from human body to plant roots) that can transfer the bacterio-siderophores in the form of phyto-siderophores to plant roots tissues [21–23]. So, the iron oxides from magnetic fluid composition (Fe\(\text{3O4}\)) can represent a source of both types of iron ions for the plant development.

The magnetic nanoparticles supplied in this experiment to plant culture medium have the mean diameter of about 10nm, the size of nanoparticles ranges between 3.75 and 21.75nm, which is suggesting the ability of small diameter ones to pass through
bio-membranes; the largest particles could remain embedded in bio-membranes or in the cell cellulose wall, so that their superparamagnetic properties could influence locally the transmembrane ion flows (magnetic influence on the ion channels).

Also, since a presumption of magnetic fluid supply interference with the nucleic acid biosynthesis is needed, one could imagine that the ferrophase could penetrate the nuclear membrane but the existence of extra-nuclear DNA and RNA need to be also taken into account. In this frame, the DNA from the chloroplasts is the most probable target of magnetic fluid effect in this experiment. Experimental investigations with germinated seeds in the magnetic fluid presence revealed that the magnetic fluid addition was able to induce cyogenetically changes, i.e. chromosomal aberrations and perturbation of the proliferation capacity [24]. Recently, in their study Gonzalez et al. [25] showed that the biocompatible magnetic fluids can be uptaken into whole living plants and further can move inside using the vascular system being concentrated in specific areas by application of magnetic gradients. In the hypothesis that large agglomerates of iron oxide particle occurs that can not be uptaken by the plant cells there still can be possible that their presence induce chronic magnetic exposure and metabolism influence at the level of various tissues.

4. CONCLUSIONS

A slow stimulatory influence of increased volume fractions of magnetic fluid diluted solution added in culture medium of plants, to photosynthesis process, was obtained for the relative low volume fractions of magnetic fluid solutions and an inhibitory effect is obvious for the highest volume fraction of magnetic fluid solution. For increasing volume fraction of magnetic fluid solution the average nucleic acid level is decreased in comparison to the control sample, revealed an inhibitory effect on biosynthesis. We might say that water based magnetic fluid addition in culture medium represented an important source of iron. But, the magnetite nanoparticles may have not only a chemical but also a magnetic influence on the enzymatic structures implied in the different stages of the photosynthesis reactions. Finally one should consider that possible bio-technological tool in the plant culture could be designed based on suitable magnetic fluid concentration range.

REFERENCES


