TMA-OH COATED MAGNETIC NANOPARTICLES INTERNALIZED IN VEGETAL TISSUE*

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The present experimental investigation was focused on the study of assimilatory pigments and nucleic acid levels in young plants intended for agricultural use (the popcorn) in presence of water-ferrofluid in culture medium. The water-ferrofluid was constituted by coating the small ferrophase particles with tetramethylammonium hydroxide and further dispersion in water. After germination, daily supply with 15 mL ferrofluid aqueous suspension per dish of sample was carried out for 12 days during plant growth. Small concentrations of aqueous ferrofluid solution added in culture medium had a stimulating effect on the growth of the plantlets, while the enhanced concentrations of aqueous ferrofluid solution induced an inhibitory effect. We have noticed that toxicity symptoms led to brown spots covering the leaf surface for the enhanced concentrations of aqueous ferrofluid 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 greatly affected leading to decrease of the metabolic process rate.

Key words: magnetite nanoparticles, popcorn plants growth, photoassimilatory pigments, nucleic acids.

1. INTRODUCTION

In recent years, substantial progress has been made in developing technologies in the field of magnetic microspheres, magnetic nanospheres and ferrofluids. Techniques based on using magnetizable solid-phase supports have found applications in numerous biological fields: diagnostics, drug targeting, molecular biology, cell isolation and purification, hyperthermia etc. [1–3].

In the last time the interest for the study of the biological effects induced by ferrofluid presence in culture medium upon vegetal organisms and microorganisms [4, 5] as well as upon animals [6] has increased. Special attention was paid to genetic effects of ferrofluids that are found to lead to chromosomal

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aberrations in young vegetal plants [7, 8] which may be related to the putative use in plant biotechnology.

Relatively small number of studies is dedicated to the influence of ferrofluids on the photosynthesis [4, 9–10] revealing the stimulatory ferrofluid effect 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 [11–12]. The iron oxides from ferrofluid composition can be a source of iron for the plant development on a ferrofluid supplemented medium. It is assumed that siderophores biosynthesis [13] can be stimulated by the ferrous and ferric iron from ferrofluid ferrophase. The biological interest in the ferrofluid 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 tetramethylammonium hydroxide on the growth of popcorn (*Zea mays*) plants in early ontogenetic stages.

2. MATERIALS AND METHODS

The present experimental investigation was focused on the study of assimilatory pigments and average nucleic acid levels in young plants intended for agricultural use (the popcorn) in presence of water-ferrofluid in culture medium. The water-ferrofluid was constituted by coating the small ferrophase particles with tetramethylammonium hydroxide and further dispersion in water. Saturation magnetization of our ferrofluid was of 10 kA/m; the ferrophase volume fraction was of 1.5% while the average particle diameter was equal to 7.98 nm.

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 40 seeds) in darkness and suitable temperature.

After seeds germination, daily supply with 15mL ferrofluid aqueous suspension per every dish with test plantlets was carried out for 12 days, plant growth being conducted in controlled conditions of temperature (24.0 ± 0.5°C), illumination (dark/light cycle: 14h/10h) and 90% humidity into a climate room. Water-ferrofluid was added daily in different concentrations (10 – 50 – 100 – 150 – 200 and 250 microL/L) in the culture medium of popcorn plantlets from different test dishes.

After 12 days of young popcorn plant growth the Meyer-Berthenrath's method modified by Stirban [14] was applied to assay the chlorophyll a, chlorophyll b and total carotenoid pigments while Spirin's method [15] 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 Perkin-Elmer 550S spectrophotometer UV-VIS provided with quartz cells. Three repetitions of assimilatory

pigments extraction and spectrophotometric assays were carried out for all experimental variant samples, average values, standard deviations and *t*-test have being considered for statistically analysis.

Plant individual length was measured with 0.1 cm 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.

3. RESULTS AND DISCUSSIONS

We have noticed that toxicity symptoms led to brown spots covering the leaf surface for the enhanced concentrations of aqueous ferrofluid solution added by us in the plants culture medium in this experiment (Fig. 1). The iron excess treatment is believed to generate oxidative stress in leaf cells. In this case, photosynthesis may be greatly affected leading to decrease of the process rate.

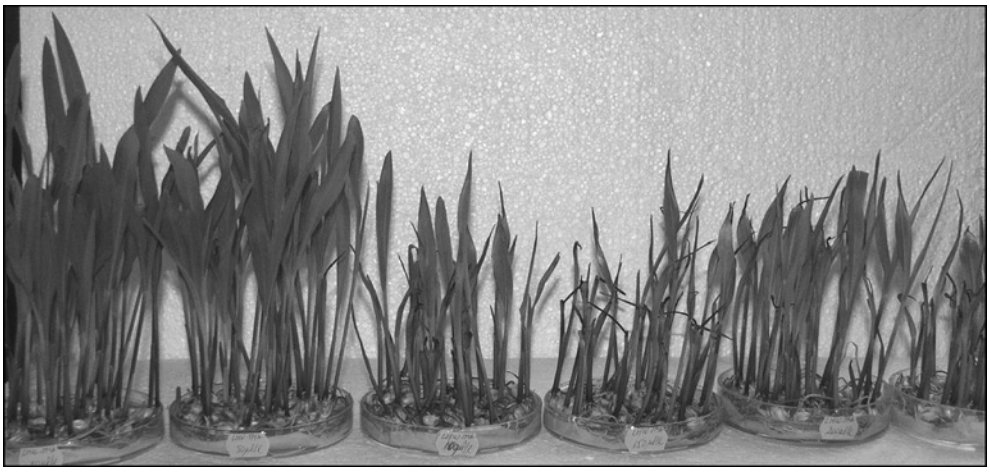

Fig. 1 – Toxicity symptoms on leaf surface of 12 days old *popcorn* plantlets.

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 aqueous ferrofluid solution supplied to the test samples.

We found that small concentrations of aqueous ferrofluid solution added in culture medium have a stimulating effect on the growth of the plantlets, while the enhanced concentrations of the same aqueous ferrofluid solution have an inhi-

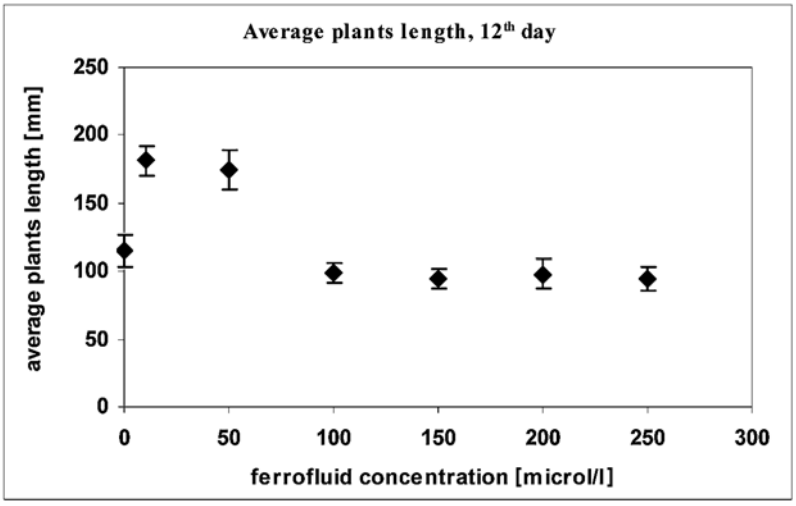

Fig. 2 – The average length *versus* concentration of aqueous ferrofluid solution added in culture medium.

bitory effect. All results are statistically significant, as resulted from the average comparison with the lengths of the control, using the Student *t*-test.

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

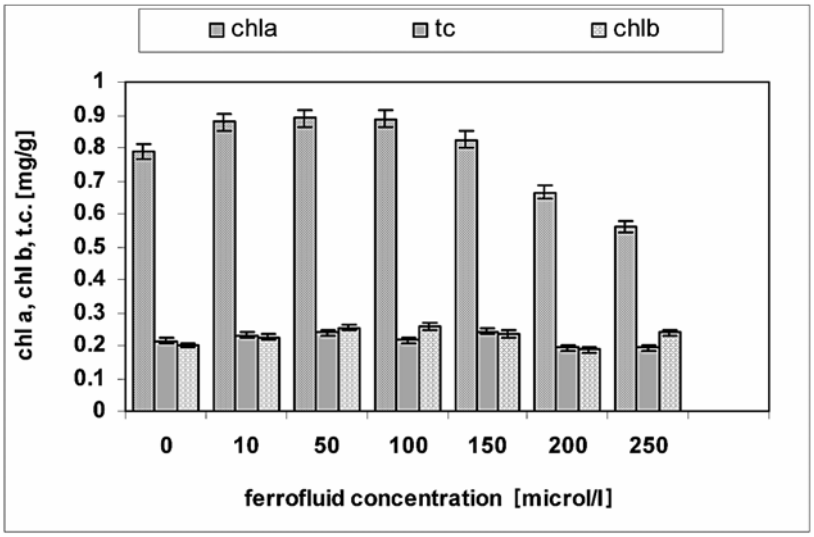

Fig. 3 – Assimilatory pigments level in *Zea mays* plantlets *versus* ferrofluid concentration (chl a – the content of chlorophyll a; chl b – the content of chlorophyll b; t.c. – the content of total carotenoid pigments).

The chlorophyll **a** level, the main photosynthesis pigment, was found increased for small ferrofluid concentrations (of 13% than control sample) while for enhanced ferrofluid concentrations added in culture medium an inhibitory effect (of 35 % compared to the control sample) was noticed. Similar response was get for the other two pigments analyzed.

The total assimilatory pigments contents (Fig. 4) have the same variation to the increase of ferrofluid concentration added in the culture medium of young plantlets that was observed for chlorophyll **a** level.

The best indicator upon the photosynthesis process efficiency is considered the chlorophylls ratio (chlorophyll **a** / chlorophyll **b**) [16] which provides indirect information on the enzymatic aggregates of the **L**ight **H**arvesting **C**omplex II (**LHC II**) from the photosynthetic system II located in the chloroplasts membranes.

In Fig. 5 an inhibitory influence of ferrofluid concentration to photosynthesis process, as suggested by chlorophyll **a** and **b** ratio, can be seen for the all ferrofluid concentrations added in culture medium of young plants; remarkable inhibitory effect is obvious for the highest ferrofluid concentration (about 35% diminution in comparison to the control sample). This can be taken as a conclusive proof of the capacity of the water-ferrofluid to influence the LHC II enzyme system.

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 ferrofluid influence except for the sample supplied with 10 microl/l ferrofluid concentration.

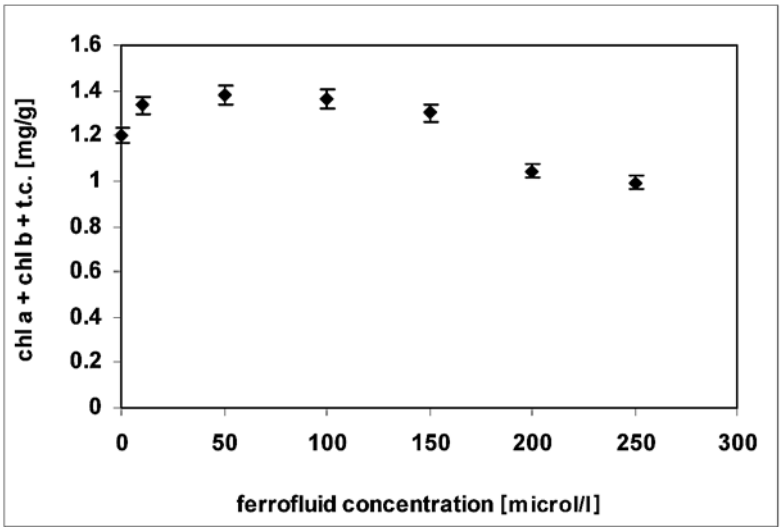

Fig. 4 – Total contents of assimilatory pigments level in *Zea mays* plantlets *versus* ferrofluid concentration.

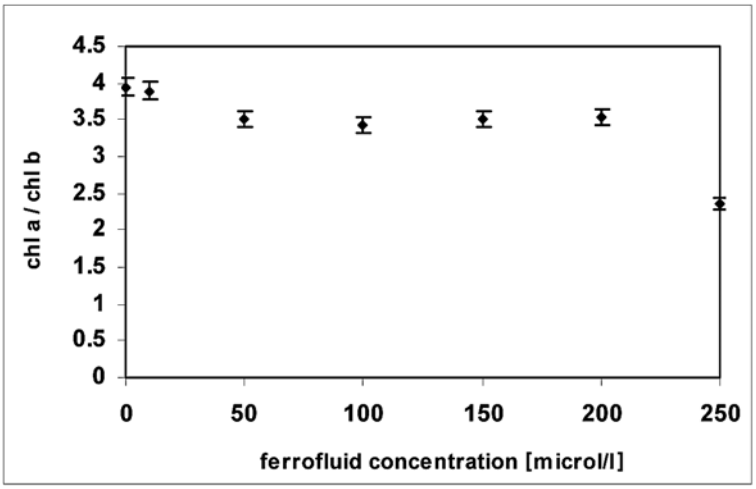

Fig. 5 – The effects of water-ferrofluid concentration added in culture medium on chlorophylls ratio.

The average content of nucleic acids in young *popcorn* plantlets after 12 days of grown under different ferrofluid concentrations added in culture medium is presented in Fig. 6.

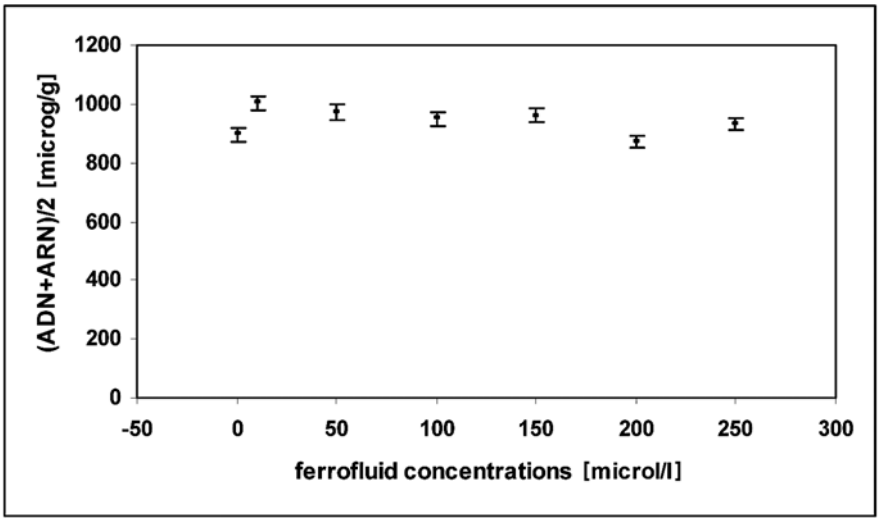

Fig. 6 – The average nucleic acid levels in *Zea mays* plantlets.

One can see that for increasing ferrofluid concentration the average nucleic acid level is slightly enhanced (about 10%) in comparison to the control sample.

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 ferrofluid influence, except the sample supplied with 200 microl/l ferrofluid concentration.

We believe that the iron oxides provided by the magnetite from ferrofluid ferrophase could interfere with the complex redox reactions involved in the photosynthesis phenomenon. More, the iron uptake in the form of iron chelates, known as phyto-siderophores, is another supposition that could be invoked when the ferrofluid influence on the photosynthesis is discussed since the putative siderophore presence in the tylakoidal membranes could result in some changes during the biochemical reactions from the vegetal cells. The iron excess treatment is believed to generate oxidative stress in leaf cells. Other scientific studies have reported that iron treatment of maize induced ferritin protein accumulation in roots and leaves over a period of 3 days [17].

TMA-OH coated magnetic nanoparticles diameter was around 8nm, suggesting the ability of bio-membrane penetration; or they could remain embedded in biomembranes 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 ferrofluid 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 ferrofluid effect in this experiment. Previous experiments with 2–3 days old germinated seed revealed that the ferrofluid addition was able to induce cytogenetically changes, i.e. chromosomal aberrations and perturbation of the proliferation capacity [18].

4. CONCLUSIONS

Relatively small ferrofluid concentrations (10–50 microl/l) may induce the increase of chlorophyll **a** level, the main photosynthesis pigment, up to 13% as well that the nucleic acid level, up to 10%, in maize plantlets during their first days of life. Higher ferrofluid concentration (100–250 microl/l) may have severe disruptive effects such as the chlorophyll **a** level and the ratio chlorophyll **a**/ chlorophyll **b** (about 35% decreasing in both cases).

We might say that water based ferrofluid addition in culture medium represented a source of iron. But, the ferrophase 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 biotechnological tool in the plant culture could be designed based on suitable ferrofluid concentration range.

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