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WATER BASED MAGNETIC FLUID IMPACT ON YOUNG PLANTS GROWING

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Abstract. The effect of a water-based magnetic fluid was tested on two plant species (pumpkin and maize) in their very early ontogenetic stages. The magnetic fluid biocompatibility was assured by the ferrophase structure (magnetite) as well as by its coating molecules (citric acid) – these molecules being well tolerated by living bodies. The magnetic fluid volume fractions used in experiment were of 10; 50; 100; 150; 200 and 250 microliter magnetic fluid per liter of water. The biochemical parameters measured in the plant green tissue were the photosynthesis pigments and the nucleic acids. Significant plant response was revealed at the level of the ratio chlorophyll a/chlorophyll b suggesting the magnetic fluid influence on the Light Harvesting Complex II from the plant chloroplasts. Different sensitivities of the two plant species to the magnetic fluid administration in culture medium have been revealed.

Key words: magnetite nanoparticles, *Cucurbita pepo*, *Zea mays*, chlorophylls, nucleic acid levels.

1. INTRODUCTION

Though known since decades the magnetic fluids (ferrofluids) still represent modern advanced materials with well-established technical applications and promising new utilizations in the life sciences. In recent years, substantial progress has been made in developing technologies in the field of magnetic microspheres, magnetic nanospheres and magnetic fluids. 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 [1–3]. Relatively small number of studies is dedicated to the influence of magnetic fluids on the plant organism: Corneanu *et al.* revealed the stimulatory magnetic fluid effect [4] on the starch accumulation in the vegetal cell (TEM investigations) while Godeanu *et al.* evidenced some stimulatory effects on

the plant growth [5]. Following such as studies for *Mammilaria duwei* [6] cultivated in culture medium supplemented with magnetic fluids (water and petroleum based magnetic fluids), the authors have observed that the metabolic activity in living tissues was much increased. Thus, the magnetic fluid utilizations have been lead at vitality of senescent tissues, decreasing of necrosis process and acceleration of springing process. The interest for the study of the biological effects induced by magnetic fluid presence in culture medium upon vegetal organisms and microorganisms [7–8] as well as upon animals [9] has increased. Special attention was paid to genetic effects of magnetic fluids that are found to lead to chromosomal aberrations in young vegetal plants [10–11] which may be related to the putative use in plant biotechnology. Considering the importance of photosynthesis (not only for the biosphere but for the whole life on Earth) and the omnipresence of iron and iron oxides in the environment, new research projects focused on magnetic fluid influence in plants are needed. The main molecular and cellular phenomena that could be taken as basic statements for the hypothesis that magnetic fluid can stimulate the plant metabolism, are the existence of an efficient mechanism of iron acquisition by graminaceous plants (resulting in the release of iron complex compounds [12] called phyto-siderophores) and the cooperation plants-microorganisms, the plants being the beneficiaries of the presence of some growth stimulatory bacteria (since under iron-limited conditions [13], these microorganisms can produce bacterial, siderophores, that can be further internalized by plant root cells). In this context, the peculiar ability of the fungus *Rhizopus arrhizus* to produce the siderophore named rhizoferrin was searched by Yehuda et al. [14] who focused on the mechanisms by which some graminae species utilize iron from phytosiderophores. We mention the results of Sherker *et al.* [15] who studied the phytosiderophores produced by plants that are excreted directly to the rhizosphere; iron uptake by barley and corn plants grown in nutrient solution was found to run parallel to the diurnal rhythms of phytosiderophore releasing via an indirect mechanism of ligand exchange between the ferrated microbial siderophores and phytosiderophores, which are then taken up by the plant.

2. MATERIALS AND METHODS

The water-magnetic fluid was composed by magnetite, prepared by iron oxides co-precipitation from auto-catalytic reaction of ferrous ($FeSO_4$) and ferric salts ($FeCl_3$) in alkali medium in accord with [16], stabilized with citric acid according to [17]. Ferrophase average diameter was of 7.8 nm, the saturation magnetization of 2.4kA/m and the ferrophase volume fraction in magnetic fluid was of 1.5%. Aqueous magnetic fluid volume fractions used in this experiment were of: 10; 50; 100; 150; 200 and 250 microliter magnetic fluid per liter of water (μl/l) resulting in ferrophase concentrations of: 0.45-2.25-4.5-6.75-9.0-11.25 μ/ml.

The biological material was provided by two plant species of agricultural interest: the popcorn (*Zea mays*) and the pumpkin (*Cucurbita pepo)*. Each sample was compound of 50 seeds, all chosen from the same plant in order to diminish the putative genophond variations. Seed germination was accomplished on watered porous paper support in glass dishes, in darkness at 22 ± 0.5ºC. After germination, daily supply with 7ml magnetic fluid aqueous suspension, for each dish, was carried out for 12 days, plant growth being conducted in controlled conditions of temperature (23 ± 0.5ºC), moisture levels being 90% and illumination (dark/light cycle 14h:10h) into a climate room Angelantoni Scientifica. Control samples were supplied only with deionized water during the experiment.

After 12 days of plant growth spectrophotometric assays were accomplished: the content of chlorophyll a and chlorophyll b and total carotenoid pigments (following the Lichtenthaler & Welburn's method [18]) and the amount of nucleic acid level (Spirin's method [19]). The spectral device was a JASCO V-530 spectrophotometer UV-VIS provided with quartz cells. Green tissue, from each sample respectively control sample, was weighted, crushed, mixed with determined volumes of acetone 80% in deionized water, for the assimilatory pigments determinations and 6% perchloric acid for nucleic acids determinations, being further quantitatively transferred into quoted glass tubes.

Assimilatory pigment assays were carried on five repetitions corresponding to each test sample and control sample. Average values, standard deviations and t-test have been considered. Student *t-test* (two tailed, pair type) was applied to evaluate reliability of modifications induced by magnetic fluid addition in culture medium to magnetic fluid supplied samples in comparison to the control ones.

3. RESULTS AND DISCUSSIONS

The contents of photosynthesis pigments (chlorophyll a, b and total carotenoids) in the green tissue of young plantlets (aged of 12 days) belonging to the two analyzed plant species in Figs. 1–6 is presented. Accordingly to Fig. 1, in pumpkin, the chlorophyll a – the main photosynthesis pigment - is increased for the smallest magnetic fluid volume fraction (10μl/l) with about 16% in comparison to the control, the statistic significance being related to the significance threshold of 0.05. For chlorophyll b content the same increase was observed, statistic significance being ensured in relation to the thresholds of 0.01 (for the magnetic fluid volume fraction of 100 μl/l) and 0.05 (for the magnetic fluid volume fraction of 10 μl/l). No significant changes could be detected for carotenoids pigments. The chlorophyll *a* content was diminished in popcorn (Fig. 2) for all magnetic fluid volume fractions (for the lowest volume fraction the inhibitory effect upon the chlorophyll a is of 40 % statistically significant in relation to the threshold of 0.01).

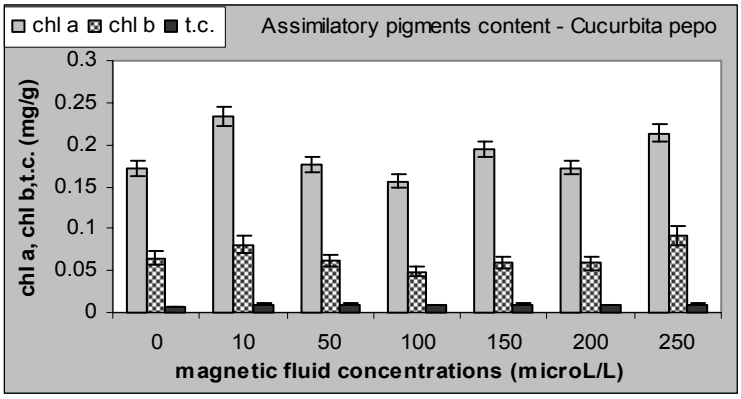

Fig. 1 – Assimilatory pigments in pumpkin (*Cucurbita pepo*) plantlets.

An exception was noticed: the sample corresponding to the volume fraction of 200 µl/l, in which slight stimulatory effect was noticed (15 % increase – statistically significant in relation with the significance threshold of 0.05). Similar response was get for the other two pigments. Linear correlation was evidenced between chlorophyll a and carotenoid pigments (Fig. 3), the correlation coefficient, R^2, being over 0.98. As known, the role of carotenoid pigments is to sustain the photosynthesis 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 (these is resulting in the biosynthesis of saccharides, proteins and lipids).

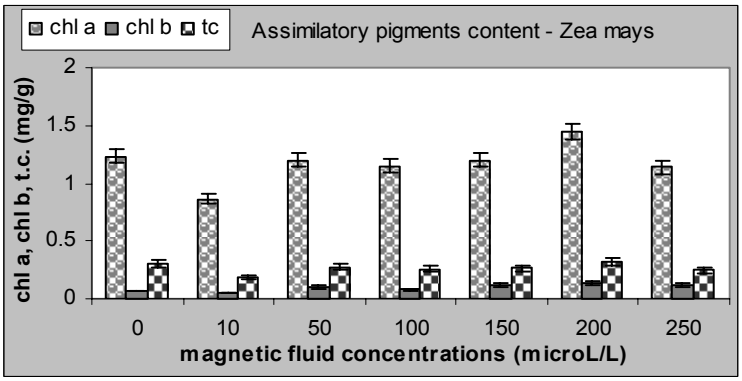

Fig. 2 – Assimilatory pigments in popcorn (*Zea mays*) plantlets.

The total assimilatory pigments contents have been slightly increased (25%) in comparison with control sample, in pumpkin, for the lowest magnetic fluid volume fraction tested in the frame of this experiment (Fig. 4).

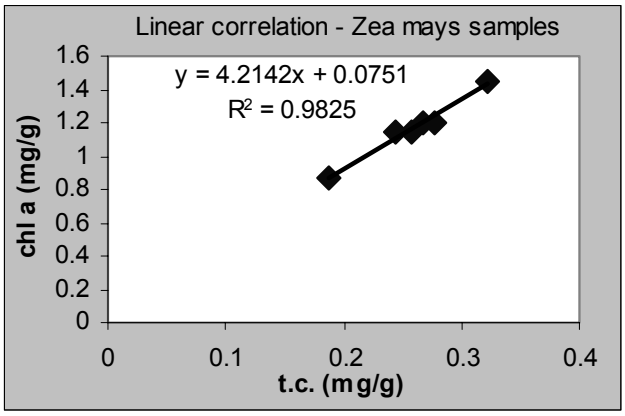

Fig. 3 – Linear correlation between the chlorophyll a and the carotenoid pigments in popcorn (*Zea mays*) plantlets treated with different magnetic fluid volume fractions.

Slight stimulatory influence was also evidenced for the volume fraction of 250 μl/l (18%). The popcorn plantlets sensitivity seems to be different (Fig. 5) as the magnetic fluid lowest volume fraction (10 μl/l) has induced a remarkable inhibitory effect upon the biosynthesis of the pigments involved in the solar energy chemical conversion (total pigment sum was decreased with 35%); however slight stimulatory influence was evidenced for the volume fraction of 200 μl/l (22%). For both these samples the statistical significance related to the significance threshold of 0.05 was ensured while non significant changes were noticed for the other samples.

The best indicator upon the photosynthesis process is considered the ratio chlorophyll a/chlorophyll b [20] 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. 6 the generally stimulation of photosynthesis process, as suggested by chlorophyll a and b ratio, can be seen for the pumpkin plantlets supplied with magnetic fluid aliquots, but a slight inhibitory influence was observed for the highest magnetic fluid volume fraction (250 μl/l) used in plant growth treatment. Statistically significant differences in comparison to the controls were noticed for the samples corresponding to the magnetic fluid volume fractions of 10 μl/l and 100 μl/l (statistical significance threshold of 0.05) while non-significant differences were found for the other samples. Comparatively the popcorn response (Fig. 7) was consistent with the approximately logarithmic decrease of chlorophylls ratio up to 50% when the magnetic fluid volume fractions increased from 10 to 250 μl/l (statistical significance was ensured relatively to the threshold of > 0.05).

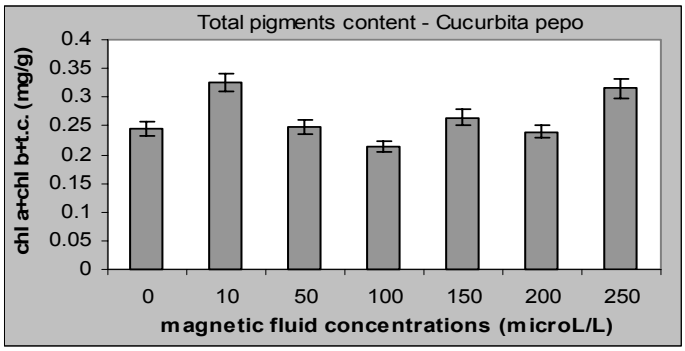

Fig. 4 – The total level of photosynthesis pigments in pumpkin (*Cucurbita pepo*) plantlets.

This can be taken as a conclusive proof of the capacity of the water based magnetic fluid to influence the LHC II enzyme system. The sensitivity of the LHC II complex to external as well to physiological factors was expected in respect to theoretical considerations upon the photosynthesis molecular basis [21–22].

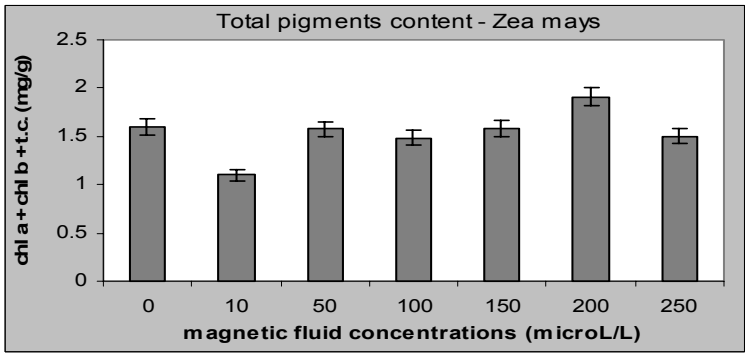

Fig. 5 – The total level of photosynthesis pigments in popcorn (*Zea mays*) plantlets.

As known the photosystem II complex is composed of more than fifteen polypeptides and at least nine different redox components (chlorophyll a, chlorophyll b, pheophytin, plastoquinone, Mn, Fe, cytochrome b559, b6, carotenoids), some of them containing ferric ions in their active sites, being shown to undergo light-induced electron transfer. But, the photosystem II reaction centers contain a number of redox components with unknown function. An example is the cytochrome b559, a heme protein, which is an essential component of all photosystem II reaction centers (discussed by Whitmarsh and Pakrasi, 1996) [22]. The photosystem I catalyzes the oxidation of plastocyanin, a small soluble Cu-protein, and the reduction of ferredoxin, a small FeS protein. Also, FeS centers serve as electron carriers in photosystem I and, as so far is known, photosystem I electron transfer is not coupled to proton translocation.

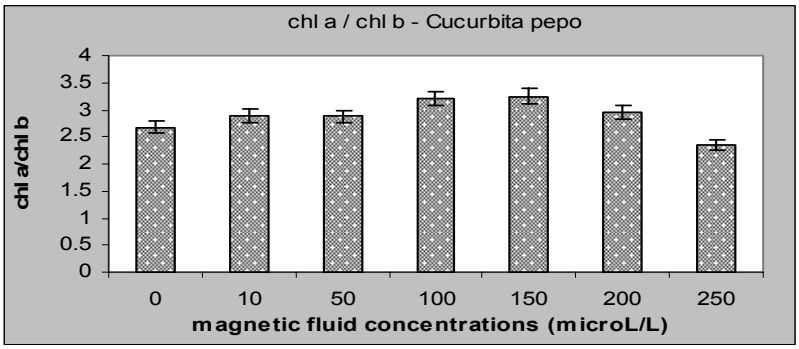

Fig. 6 – Magnetic fluid effect on the chlorophylls ratio in pumpkin (*Cucurbita pepo*) plantlets.

Consequently the iron oxides provided by the magnetite 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 magnetic fluid influence on the photosynthesis is discussed since the putative siderophore presence in the tylakoidal membranes could result in some changes in the biochemical reactions from the vegetal cells.

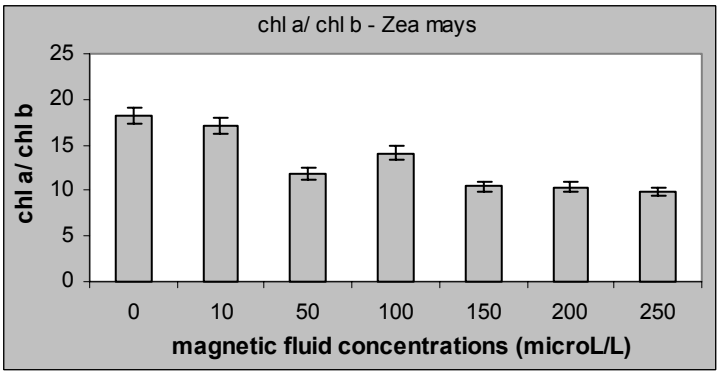

Fig. 7 – Magnetic fluid effect on the chlorophylls ratio in popcorn (*Zea mays*) plantlets.

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. Indeed, though the ferrophase diameter was adjusted as small was possible during the chemical precipitation reaction (known for the difficulty of particle size control) however certain larger particles or aggregates could not be able of bio-membrane penetration and thus could remain embedded in them or in the cell cellulose wall so that their superparamagnetic properties could influence the transmembrane ion flows (magnetic influence on the ion channels).

Further, the intra- and inter-cellular communication could be locally affected leading to the indirect influence on the photosynthesis control; the plant response to such low level stimuli is expected to be dependent on the species since the enzymatic equipment which is very sensitive (due to the mobility of electron and proton involved in the redox chains) may differ in some respects among the plant species. In Fig. 8 the content of DNA and RNA for the pumpkin plantlets is presented following the magnetic fluid addition in different volume fractions. One could say that in pumpkin there are no coherent changes while in popcorn a slight inhibitory effect was noticed (Fig. 9).

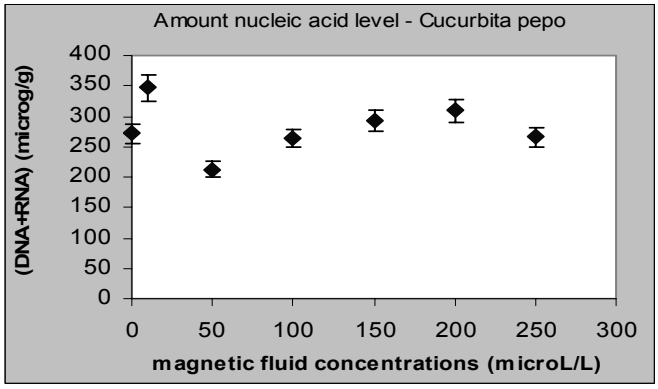

Fig. 8 – The amount nucleic acids level for the pumpkin (*Cucurbita pepo*) plantlets under magnetic fluid influence.

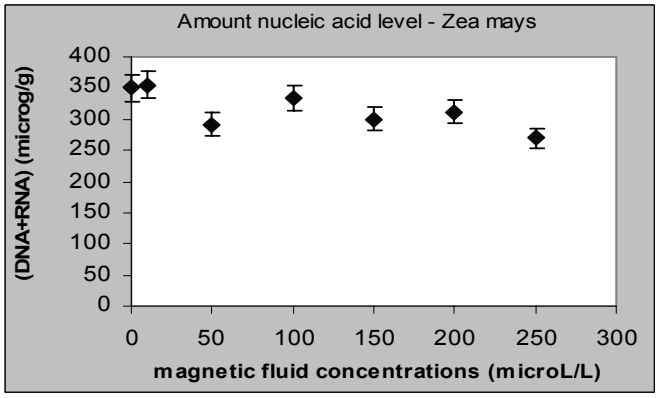

Fig. 9 – The amount nucleic acids level for the popcorn (*Zea mays*) plantlets under magnetic fluid influence.

So, 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 may focus also on the existence of extra-

nuclear DNA and RNA. In this frame, the DNA from the chloroplasts is the most probable target of magnetic fluid effect in this experiment – considering also the similitude between the magnetic fluid inhibitory effect on the nucleic acid and that on the LHC II from the chloroplasts. Previous experiments with 2-3 days old germinated seed revealed that the magnetic fluid addition was able to induce cytogenetic changes, i.e. chromosomal aberrations and perturbation of the proliferation capacity [10–11]. So, finally one should consider that possible biotechnological tool in the plant culture could be designed based on suitable magnetic fluid concentration range.

4. CONCLUSIONS

The water-magnetic fluid added in the deionized water supplied during the first 12 days of growth of pumpkin plants has resulted in slight stimulation of chlorophyll ratio while in popcorn logarithmical diminution of chlorophyll ratio was evidenced. The average content of nucleic acids was slightly diminished in the same popcorn plants. The sensitivity to the given magnetic fluid (composition and concentration) seems to be dependent on the plant species. Further investigation on other plant species, with other biocompatible magnetic fluids (such as containing magnetite core- β-ciclodextrin shell) and involving complementary investigation methods are planned aiming to get new information on the photosynthesis sensitivity to magnetic fluids.

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