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Magnetic fluids improving effect in in vitro regenerates in hypogravity conditions

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Abstract

The previous in vitro experiments done with different types of magnetic fluids (MFs) pointed out the favorable effect upon dedifferentiation and redifferentiation. Finally, regeneration of an embryo and of plantlets from a single vegetative plant cell was successfully performed. The two processes are the most spectacular aspects of development involving the formation of different distinct cells and of tissue which increase the potential for variability to new conditions. These advancements generated the idea that the MFs could have a favorable effect on cell morphology and tissue structural organization. This study addressed the question of whether or not Magnetic Fluids could have a protective effect on plants grown under different stress conditions as well as in simulated hypogravity influence. In simulated hypogravity two possible patterns of influence were postulated: reversible and irreversible cell modifications. The presence of the magnetic particles (MPs) in the growth medium improved the cell and the anatomical leaf structure. The favorable effect was the enrichment of the density, due to MPs penetration in cytoplasm, or due to the change in the cell water potential. We presume both. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Simulated hypogravity; Damages of FMs improving; Anatomo-histologicall structure; In vitro growth

1. Introduction

Gravity affects the orientation of plants in many ways [1]. It is unclear whether each cell senses gravity, or specialized cells (statocytes) have the ability to sense it. The starch grains present in amyloplasts are considered to have the ability to perceive gravity [2,3]. There is experimental evidence that the cytoskeleton is involved in the gravity perception mechanism [4,5]. Evidence also exists which suggests that actin and tubulin are not altered [6]. Reduced gravity caused striking effects on the cell morphology and leaf structural organization [7,8].

The previous observation revealed the magnetic fluids (MFs) beneficial effect in regeneration and in

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plant growth [9]. To know whether the MFs can have a favorable effect upon plant cells and tissues also in hypogravity conditions, we studied the anatomical leaf structure of in vitro tobacco plants.

2. Experimental conditions

2.1. Plant material and growth conditions

In vitro neoplantlets obtained from an explant of the Nicotiana tabacum Imun variety grown in B5 normal growth media [10], supplemented with water-based magnetic fluids (B5-FM) were used. The leaf tissue structure was analyzed after continuous simulated hypogravity conditions (24 days). The last completely formed leaf was the subject of microtome semithin and ultrathin sections. In the different treatments the leaf aspect was particular: light green in control, lighter with a necrotic tiny border in hypogravity, and a healthy, normal dark green in hypogravity cultivated in media supplemented with MFs. The specimens were prepared for sectioning by being fixed postfixed, dehydrated and then embedded in Epon 812. The specimens were then processed according to electron microscopy techniques [11,12].

The in vitro plants grew in different experimental variants (V): (A) terrestrial gravity in B5 normal media (Control, V1); (B) simulated hypogravity in: B5 normal growth media (V2), B5 supplemented with MF having $\theta = 1.15 \times 10^{-3}$ g/cm^3 magnetic particles (MPs) (V3) and B5 supplemented with MF having $\theta = 15 \times 10^{-3}$ g/cm^3 MPs (V4). Fe_3O_4 and/or γ-Fe_2O_3 magnetic particles precipitated in alkaline medium were used. Stable water-based magnetic fluids, up to 20 kA/m magnetization were prepared using dodecylbenzene–sulphonic (DBS) acid as primary (chemisorbed) and secondary (physically absorbed) surfactant [13].

2.2. The clinostat parameters

In the experiments described in this paper, a clinostat was used to simulate hypogravity condition. The clinostat is a rotating machine with a horizontal axis of rotation. A plant well attached and centered over the rotor of the device experiences a 1-g stimulus at every moment within the plant body, but this stimulus changes direction once at each revolution. It is supposed that the plant senses all these multiple stimulation which sum, to zero for any cell at complete revolution. The rotation speed of the rotor was slow in order to keep the centrifugal acceleration below 2×10^{-5} g.

3. Results and discussions

The leaf is the main photosynthetic organ of the plant. It is an outgrowth from the stem. It comprises the lamina and its blade is flattened. Leaves of tobacco plants grown in vitro formed a typical leaf blade (V1). It consisted of a normal epidermis, a uniform cell line of weak palisade parenchyma and well-formed mesophyll and conducting tissues. The mesophyll is composed of more or less elongated cells arranged in radial columns. The spongy mesophyll formed the ground tissue in the leaves.

In control (V1) the tissues and each type of cell were almost normally structured (Fig. 1a). The cell components: nucleus, nucleolus and, the chloroplast and the mitochondria were all normally organized.

In simulated hypogravity conditions (V2) the leaf structure was dramatically changed (Fig. 1b). The epidermal stomata guard cells were closed which is an unusual behavior for in vitro culture. The bulk part of mesophyll was composed of variously shaped cells, often conspicuously lobed of angular with large intercellular spaces. It is sometimes regarded as an aerenchyma type. The majority of the cells were destroyed. At the tip of the leaf almost all cells were dead. In the rest of the tissue the cells were empty. Only in some of them was the cytoplasm present. The nuclei and nucleolus were well developed in the majority of the cells. With a marginal positioning, they were modified in some of the cells. The gradual modification of the cells occurring along the leaf blade indicate less modifications in the basal and middle part of the leaf in comparison to the top one. The small size chloroplast could not be observed with the light microscope. The lamina was thicker as in the control but with a changed electron density. It was larger, indicating the degree of its modification. Between laminas

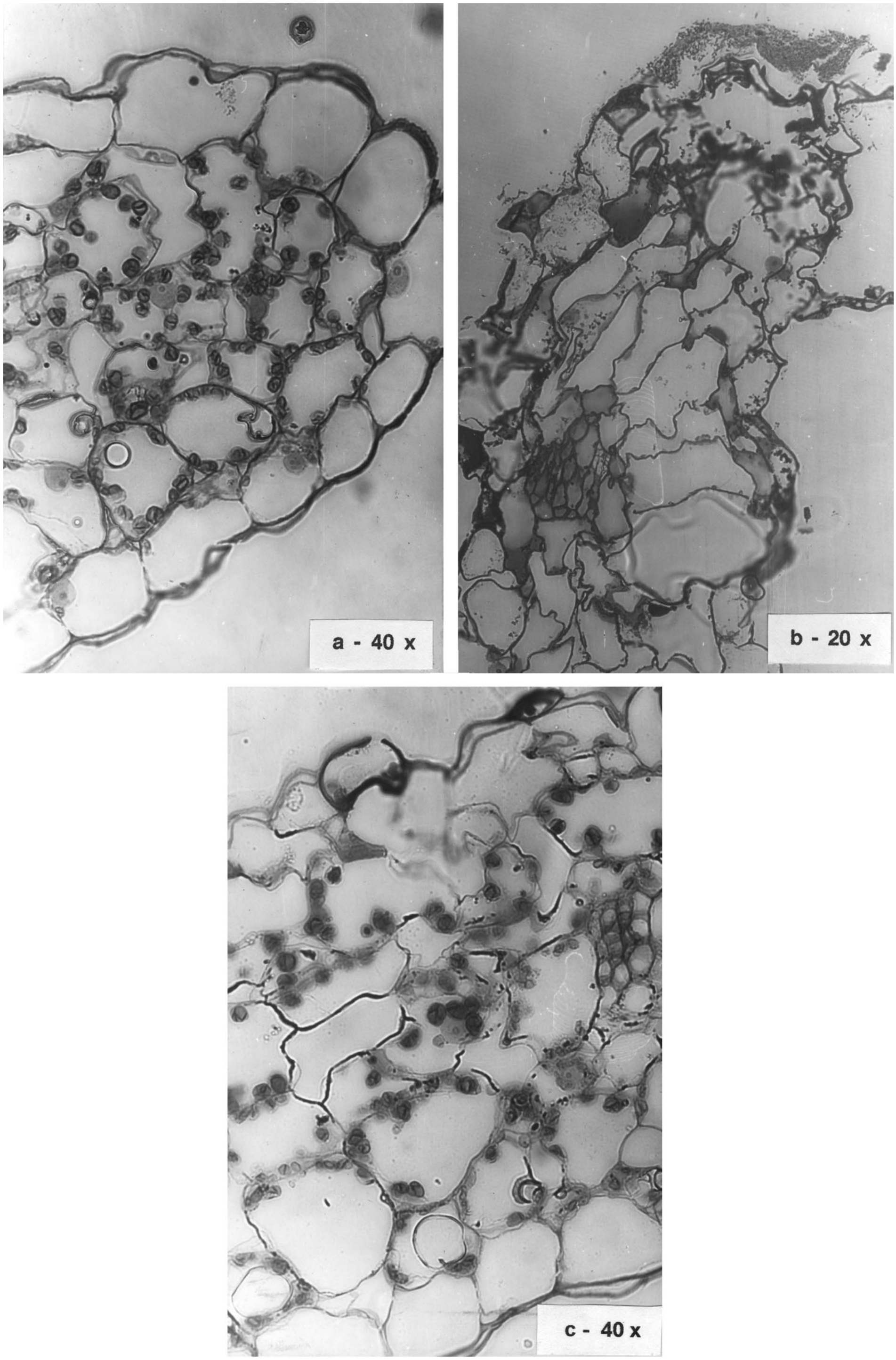

Fig. 1. The general view of anaomo -structural leaf assembly of Nicotiana tabacum, L. grown in in vitro in terrestrial gravity (a), simulated hypogravity (b) and in the presence of MPs in simulated hypogravity (c). (a) Normal aspect of mesophyll cells in tobacco grown in terrestrial gravity. (b) The obvious changes in the cell structure of leaf mesophyll grown in simulated hypogravity. (c) MPs $\theta = 1.15 \times 10^{-3}$ g/cm^3 as an improving factor for simulated hypogravity.

there were large spaces revealing the cell separation, which gave the intercellular separation and finally the tissue degradation.

For the plant growth and cell structure/organization in hypogravity some possible patterns of influence were considered: (1) little or no response such as nucleus and nucleolus, (2) with a reversibility potential as, for instance, the fluid part of the cell and, (3) irreversible modifications as in the chloroplast, mitochondria, and the membrane system.

The favorable effect of MFs of the leaf's anatomical structure was obvious having a high significance when the MPs concentration was $\theta = 1.15 \times 10^{-3}$ g/cm^3 (Fig. 1c). In the presence of a small quantity of Magnetic Particles (MPs; $\theta = 1.15 \times 10^{-3}$ g/cm^3), the leaf blade cells were like those in the control. In FM-hypogravity variant the epidermis and the cuticle were almost normally shaped. However, the epidermis was interrupted by stomata, usually more numerous on the leaf axial (lower) side than in the control. Some stomata were opened but some of them were closed. The mesophyll cells were well made, expressing a slight hypertrophy. The most obvious changes were observed at the upper palisade parenchyme. The palisade parenchyme was made of heterogeneous cells making a compressed central cell zone. The hypertrophied tissue cells revealed a specific aspect. The first subepithelial tissue contained three layers: small and rounded cells formed the first, large squared cells the second, while highly elongated cells formed the third layer in a radial arrangement. These three layers made a clear median zone where the upper-surface leaf tissues met the lower one. The lower epidermis contained the subepidermis layer with rounded cells and a second one with very prolonged cells. In the median part of the leaf a very large vascular system developed. It was similar to vascular tissues but modified cells also occurred. The nuclei were rounded or slightly ovoid, with the nucleolus situated in the central part pointing out an intense activity. The nuclei were also active even when their arrangement was lateral and they were flattened onto the cell wall. The chloroplast structure was like that of the control but chloroplasts were smaller and randomly distributed in the cell. The mesophyll cells contained more chloroplasts than those of the control (V1, terrestrial gravity). Clamp conglomerate chloroplasts surrounded the nuclei in many cells.

When the concentration of magnetic particles was higher ($\theta = 15 \times 10^{-3}$ g/cm^3) the leaf tissue structure was slightly different from that of the control (V1) and of the small concentration variant (V3). There were no differences in the epidermis and the cuticles structure. The mesophyll cells were hypertrophied. The important differences consisted in the layer organization. There were only two subepithelial layers. Small and rounded cells were the first, as in V1 and V3. The second one contained very large and elongated cells randomly distributed extended to the median part of the leaf. The lower epidermis and stomata were normally made. At the transversal leaf middle, the place where the two tissue parts meet, a highly compressed median region was formed, rich in vascular bundles.

The degree or intensity of slight modification of the cell's ultrastructure produced during in vitro growth in hypogravity conditions was evidentiated through electron microscopy investigations. In the ultra view the main characteristics of chloroplast consist of strong vesication being completely destroyed. Only some of them contained small starch granules and electron-dense spots. Preliminary studies revealed the same aspects in pea [14,15] and pepper [16] leaves. The starch transportation was obstructed. The inner dark spots can be phenol drops indicating the stress condition. The most frequent effects were interthylacoidal and stromal rarefaction, peripheral swellings and the breaking of the chloroplast encasement. Many of the organelles were in junction with the endoplasmic reticulum. A few grains of chlorophyll were free in the amorphous stromal content. One or two of the endoplasmic reticulum, as in algae, surrounded some chloroplasts. The mitochondrion was almost without structure. The swollen mitochondria contained large cryistae-indicated senescence. There were no significant differences between the control (V1) and the FM-variant. The $\theta = 1.15 \times 10^{-3}$ g/cm^3 ultrastructure's assembly was better than that of the control (V1). In the MPs presence the cell and tissue heterogeny were larger. Only a few small cells with well-structured nucleus and nucleolus were present. We presume that an unequal mitotic division generated them. The chloroplasts exposed a very well-developed ultrastructure. They were free and active. In the regular spongy cells the chloroplasts were more or less uniformly distributed. In the electronic microscopic investigation, the structure of the chloroplasts was organized like an amiloplast. Many normally structured chloroplasts contained big starch grains. The large strach reserves contained in the leaves pointed out the MF improvement effect. A normal picture of the mesophyll was also presented. Large numbers of mitochondria were tightly packed together with cristae in them, which indicate an active energy level for the cell's metabolism. The ultrastructural investigation pointed out clearly that the cell's structures were improved in the presence of MFs.

4. Conclusions

The cell and the leaf tissues were significantly modified under simulated hypogravity. A graduated cell modification took place along the leaf blade. The matured cells suffered more changes than the immature ones. The chloroplasts and mitochondria beard progressive disintegration. Swollen mitochondrion appeared in hypogravity conditions pointing out premature senescence.

The following possible patterns of cell behavior in hypogravity were postulated: a decreased response of the nucleus and nucleolus, reversible modifications in the fluid part of cell and irreversible modifications in the chloroplast, mitochondria and membranes system.

The presence of Magnetic Particles in the growth medium improved the cell structure, tissues and finally the plant growth. The anatomical leaf structure was also improved. The $\theta = 1.15 \times 10^{-3}$ g/cm^3 concentration of magnetic particles in the growth medium induced the greatest improving effect.

The magnetic fluids can be used as a protective material against hypogravity stress. The positive effect consists of density enrichment due to the MPs penetration in cytoplasm or due to the change in cell water potential.

We presume both mechanisms.

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