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The effect of the non ionizing radiation on cultivated plants of *Arabidopsis thaliana* (Col.)

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ABSTRACT

A series of experiments was carried out to investigate any structural or biochemical alterations on *Arabidopsis thaliana* (Col.) plants after a long term exposure to non ionizing radiation emitted from the base unit of a cordless DECT system. Exposed plants, compared to their control counterparts, seem to be affected concerning their biomass and leaf structure. Their leaves are thinner and possess fewer chloroplasts. SEM observations of the exposed leaves reveal that the only feature affected is the pubescence which almost disappears while TEM investigation revealed minor structural effects in the chloroplasts. The reduction in the number of chloroplasts as well as the decrease of stroma thylakoids and photosynthetic pigments are probably the main reasons for a weak photosynthetic potential and a consequent reduction of the biomass production.

1. Introduction

Ionizing radiation imposes every living creature to a series of alterations, starting from the initial absorption and leading to the final biological injury. Visible light, UV, X-rays and Gamma rays are electromagnetic (EM) radiations differing in frequency and, consequently, in energy (Kovács and Keresztes, 2002; Esnault et al., 2010). Gamma rays carry a huge energy load (from around 10 keV to several hundred keV) and they are considered to be the most penetrating than other radiations or even particle emissions such as alpha and beta particles (Kovács and Keresztes, 2002). UV radiation seems to affect plant life in spite the fact that plants, having the ability to exploit sunlight, do have some means of protecting the living cells from the damages of DNA, and the membranes (Hollósy, 2002). It has also been documented, long ago, that Gamma radiation has both stimulatory and inhibitory effects upon plant growth. These effects can be both morphological and ecological and they are reflected through species interactions at the community level (McCormick and Platt, 1962). It is well documented that plant seeds are affected in the state of dormancy (Akbal et al., 2012) while the growth of plants irradiated with a high dose gamma ray (50 Gy) was significantly inhibited (Wi et al., 2007) with the parenchyma cells being more sensitive in gamma rays among the cells of any other plant tissue.

On the other hand it seems that Earth's natural radiofrequency environment has remained more or less the same within the lifespan of modern trees since before 1800, the major components of this environment were broadband radio noise from space (galactic noise), from lightning (atmospheric noise), and a smaller Radio Frequency (RF) component from the sun (Haggerty, 2010). That means that plants may have evolved to use these environmental signals, along with visible light in order to regulate their periodic functions. Therefore, they may be sensitive to man-made RF fields (Haggerty, 2010).

During the last decade, when mobile phones turned to be the most common form of communication, citizens of the civilized world thrive within a "cloud" of non ionizing radiations. The rapidly increasing use of the cellular technology resulted in an increase of electromagnetic radiations in the environment (Sharma and Parihar, 2014). Much concern is given to the effects of this radiation to human life and environmental health (Roux et al., 2006; Pietruszewski et al., 2007; Sheridan et al., 2010; Sharma and Parihar, 2014). Some concern was given to plant reactions (Ledoigt, 2006; Haggerty, 2010; Kumar et al., 2015) yet only a few data is available on the biomass production, leaf anatomy and tissue organization, overall for a single species. In this paper we present a complete series of data for a single species, *Arabidopsis thaliana* (Col.), cultured under controlled conditions in specially designed

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Table 1
Average and peak electrical field intensity, in each experimental setup, as measured for a 6 min period.

CAGE	Average	Maximum – integrated	Maximum – peak
Control	0.073 V/m	0.458 V/m	0.490 V/m
Exposed	2.072 V/m	11.320 V/m	27.460 V/m

Faraday chambers, after continuous, long term exposure to DECT radiation.

2. Materials and methods

2.1. Plant material and exposure setup

Seeds of wild – type *Arabidopsis thaliana* (Col.) (Fig. 1) were imbibed and grown at 22 °C (70% humidity) with a light/dark cycle of 16 h/8 h and 110 μmol/m^{2}s of Photosynthetically Active Radiation illumination (PAR) supplied by cool-white fluorescent tungsten tubes (Osram, Germany). Sprouts, at the cotyledon stage, were shown in 80 mm × 80 mm, 400 mL pots filled with wet potgrond P medium (Klasmann – Deilmann), at pH 6,0. Five individuals were accommodated in each pot (Fig. 2). Six pots were placed in each of the two Faraday cages (40 × 40 × 25, covered with 0.8 mm mesh – 0.1 mm stainless steel wire) thoroughly checked (spectrum analyzer: NARDA SRM3000, Germany), after their construction, for their ability to isolate any radiation emitted from within. The cages had a build–in light source (Philips, CorePro LED bulb 11.5 W equivalent to 75 W, warm white = 2700 K, 105 mA) producing 2500 lux radiation at the surface of the pots (Fig. 3).

Both cages were placed in a ventilated, adjustable temperature P-Selecta incubator (Model No. 2000238 – Barcelona, Spain) where they remained at 22 °C, for two weeks (experiment 1), three weeks (experiment 2) and four weeks, when the plants were about to complete their life cycle (experiment 3). In the middle of one of the two chambers the base unit of a DECT telephone apparatus (General, Model 123) was appropriately positioned. The DECT base was in a 24 h a day, 7 days a week pulsed transmission mode, at 1882 MHz, as described elsewhere (Margaritis et al., 2014) while the light/dark programme of the chamber was adjusted to a 16/8 cycle.

Radiation was measured in the two cages, while the DECT device was transmitting within one of them, with the NARDA SRM3000 (Germany) spectrum analyzer. The corresponding electrical field intensity (average and peak), in each experimental setup, was measured for a 6 min period according to ICNIRP (1998) guidelines as in Table 1.

Supplementary, low precision measurements were made in the control cage, with a broadband field meter (TES – 92, 50 MHz – 3.5 GHz, Electromagnetic radiation detector) at the value of 490.1 mV/m (Fig. 4, left). In the nearby cage (exposed), radiation reached the value of 27.46 V/m (27.460 mV/m, at 1882 MHz) (55 fold higher) (Fig. 4, right).

Three experiments, differing in the culture period (two weeks, three weeks and four weeks), were conducted. At the end of each culture period, the pots were removed from the cages. The plants were washed from the potgrond-P medium, placed on filter paper and left do dry at 60 °C, for three days. All the plants were weighed for their above the ground part and their root system. A small part, from the middle of the leaf, close to the central nerve, from one leaf of each plant, was cut in to small pieces (1 mm × 1 mm) and fixed in phosphate buffered 3% glutaraldehyde (pH 6.8) at 0 °C for 2 h. A few pieces were dehydrated in graded acetone series, critical point dried, coated with gold to be viewed with a JEOL JSM – 6360 Scanning Electron Microscope. The rest of the tissue was post fixed in 1% osmium tetroxide in phosphate buffer, dehydrated in graded ethanol series and embedded in Durcupan ACM (Fluka, Steinheim, Switzerland). Semithin sections obtained from a LKB Ultrotome III, were placed on glass slides and stained with 0.5 toluidine blue O (in 1% borax solution), as a general stain, for light microscopic observations. Ultrathin sections were placed on 100 mesh grids, double stained with uranyl acetate and lead citrate (Reynolds, 1963) and viewed with a Phillips EM – 300 Transmission Electron Microscope.

Fixation was repeated after each one of the three experiments and the embedded tissues were sectioned and observed, for cross-checking the results. Literature for double fixation is cited in detail by Christodoulakis et al. (2009) and Christodoulakis et al. (2010).

Table 2
Biomass values for stems and roots of the *A. thaliana* (Col.) plants in each one of the three experiments.

Dry weight of *Arabidopsis thaliana*		
Arabidopsis thaliana 1st experiment		
after 2 weeks of growth	above ground	root
control (20 plants)	52.9 ± 2.1 mg	16.8 ± 1.4 mg
exposed (20 plants)	23.5 ± 2.3 mg	4.1 ± 0.9 mg
Arabidopsis thaliana 2nd experiment		
after 3 weeks of growth	above ground	root
control (30 plants)	581.2 ± 6.2 mg	167.1 ± 4.1 mg
exposed (30 plants)	378.2 ± 4.3 mg	99.2 ± 5.3 mg
Arabidopsis thaliana 3rd experiment		
after 4 weeks of growth	above ground	root
control (30 plants)	851.5 ± 7.9 mg	197.3 ± 5.1 mg
exposed (30 plants)	725.8 ± 4.4 mg	128.2 ± 4.6 mg

2.2. Pigments protocol

Chlorophyll pigments, from an approximately 50 mg mass of leaves, were extracted with 1 mL 80% acetone, overnight, at 4 °C. Supernatant was transferred to a 1 mL glass cuvette for measurement in a UV/Vis Specol photometer (Zeiss). Absorbance was read at both 663.6 and 646.6 nm, corresponding to *chlorophyll–a* and *chlorophyll–b* respectively. Furthermore, absorbance of *chlorophyll–c* at 625 nm was also obtained. Quantification of pigment content was calculated using molar extinction coefficients specifically for this method (Gechev, 2013). Chlorophyll-a, $e_{663.6} = 76.79$ and $e_{646.6} = 18.58$; chlorophyll-b, $e_{663.6} = 9.79$ and $e_{646.6} = 47.04$. The total chlorophyll content is calculated using the following formula and normalize per fresh weight: Chlorophyll a + b = 19,54A646.6 + 8,29A663.

3. Results

3.1. Plant morphology and leaf anatomy

Five individuals of *A. thaliana* (Col.) were grown in each pot. The differences in plant development can easily be demonstrated (Figs. 5 and 6). Untreated plants, from the first stages of their life (Fig. 5), bear more and larger leaves in the rosette while the treated plants are delayed in growth (Fig. 6) and their leaves are fewer and smaller. The above ground part of all plants (treated and untreated) proved always heavier than the root. The values for the dry weigh of the above ground (stem and leaves) and the below ground (root) parts of the two groups, in each of the three experiments, are given in Table 2.

Microscopical observations of semi thin sections reveal some obvious differences in leaf thickness, development of mesophyll tissues and intercellular space formation.

The leaves from the untreated *A. thaliana* (Col.) individuals are very thin and have a rather peculiar, fragile structure with a tissue arrangement uncommon for the most dicotyledons. They have a papillate adaxial (upper) epidermis with large cells (yellow arrow in Fig. 7), a hardly distinguished palisade parenchyma with a layer

Figs. 1–4. 1. A mature, untreated plant of *A. thaliana* (Col.). 2. Three of the pots prepared to be placed in the cages. 3. The two Faraday cages during one of the experiments. The black, DECT base unit is visible within the right cage (arrow). 4. The intensity of radiation was measured in the left, control cage (0.490 V/m) and the right cage (27.460 V/m), with the exposed plants.

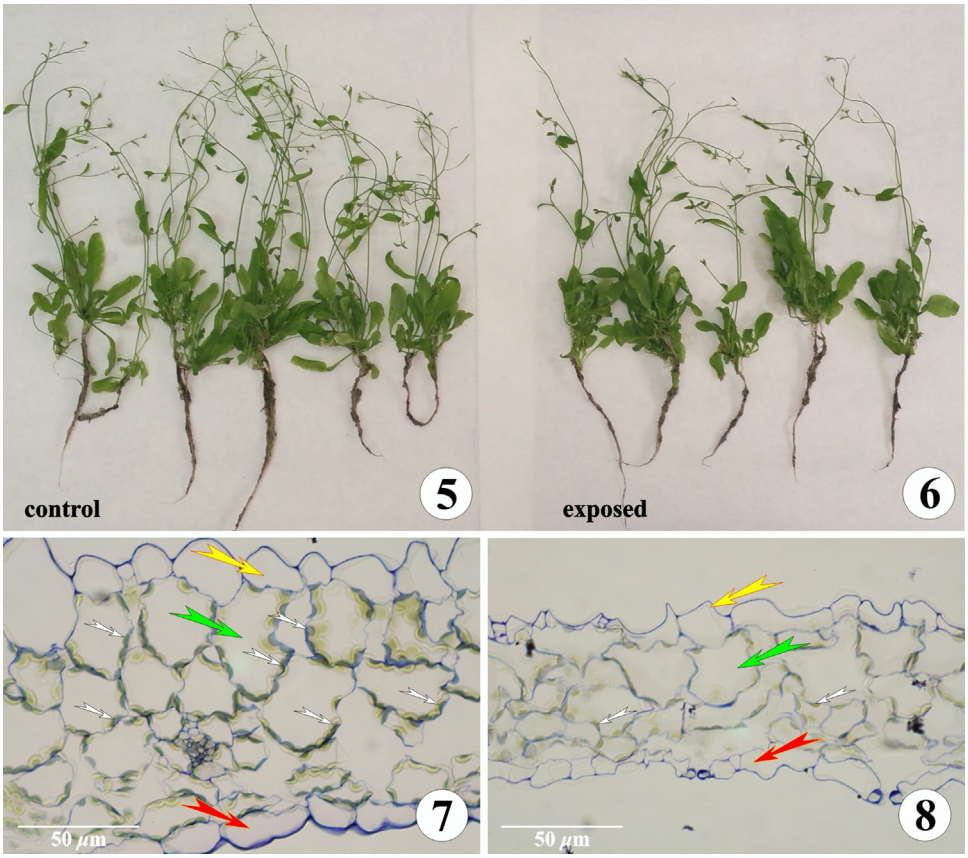
of rather bulky than elongated, palisade–like cells (green arrow in Fig. 7) and a less developed but typical spongy parenchyma. The abaxial (lower) epidermis is composed of flat cells (red arrow in Fig. 7). The outer periclinal wall of the cells in both epidermal tissues is thicker that the rest of the cell wall, thus stained more vividly (Fig. 7). Stomata appear on both surfaces. Numerous chloroplasts can be observed within the mesophyll cells (white arrows in Fig. 7). The conductive bundles, with the exception of the central nerve, are hardly developed.

The exposed leaves have about half the thickness compared to the control ones (Fig. 7). The adaxial epidermal cells seem smaller (yellow arrow in Fig. 8) with thinner walls. The same is true for the abaxial epidermis (red arrow in Fig. 8). The photosynthetic tissue arrangement is rather vague with the "palisade" cells still bulky and rounded (green arrow in Fig. 8). Finally, the number of chloroplasts within the photosynthetic cells (white arrows in Fig. 7) is far smaller to that of the untreated leaves.

The values for the five major chloroplast pigments from the control and exposed leaves are given in Fig. 9. It seems that the quantity of the pigments is affected and appears inferior in the exposed leaves. Differences between control and exposed leaves are significant for the beta–carotene, xanthophylls, chlorophyll–b and chlorophyll–a fraction whereas the differences between the chlorophyll–c fractions appear statistically ambiguous.

Scanning Electron Microscope (SEM) observations of whole mounts of leaves revealed only one major difference between control and exposed plants. Control plants appear to have many unicellular, branched trichomes on the adaxial (upper) epidermis only (Fig. 10), while the exposed plants merely bear some trichomes (Fig. 11). The abaxial (lower) epidermal cells, in both plants, are huge, multilobed and appear to have a very peculiar, interweaving linkage. It seems that epidermal cells of exposed leaves (Fig. 13) appear somehow deformed compared to the epidermal cells of the control leaves (Fig. 12). Finally, stomata, with their well preserved, fragile protrusion (edge) of their guard cells, appear similar, in both control (125 ± 12) (Fig. 14) and exposed leaves (129 ± 9) (Fig. 15).

Ultra-thin leaf sections of epoxy embedded leaves, observed with the Transmission Electron Microscope (TEM), confirm that the chloroplasts in the cells of the exposed leaves are inferior in number yet chloroplast structure, in both groups of plants, does not seem to present major differences. Chloroplasts in the cells of the exposed plants exhibit complex, well elaborated grana thylakoids yet their fretwork (stroma thylakoids) seems sparse and their plastoglobuli (arrows in Figs. 17 and 19) outrun in number the same osmiophillic droplets of the chloroplasts in the cells of the control plants (Fig. 16). In higher magnifications, stroma thylakoids and all the grana appear similar and unaffected in the chloroplasts of both plant groups (Figs. 18 and 19). Chloroplasts from the cells of exposed plants have their stroma less dense than that of their control counterparts (Figs. 16–19). Concerning mitochondria in the cells of the exposed plants, our observations indicate that they keep their membrane system undisturbed yet they appear to possess a denser matrix (Figs. 20 and 21). Nuclei appears similar, in both plant groups, while in most cells of the exposed leaves we observe myelin like structures. Sometimes these structures are huge and appear to be associated with the vacuole.



Figs. 5–8. All the plants of an experiment in the same snap-shot, to demonstrate the differences, (5) normal plants, (6) exposed plants. 7. Leaf cross section from a plastic embedded normal leaf (three- and four-week experiments). 8. Leaf cross section from a plastic embedded exposed leaf (three- and four-week experiments). Arrows point at: yellow → adaxial (upper) epidermis, green → palisade parenchyma, red → abaxial (lower) epidermis, small white → chloroplasts.

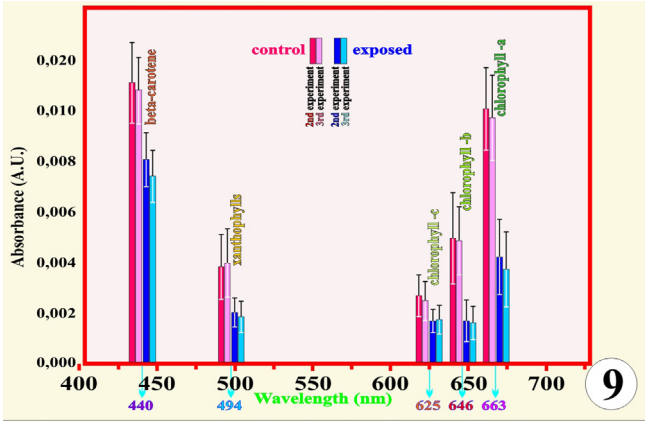


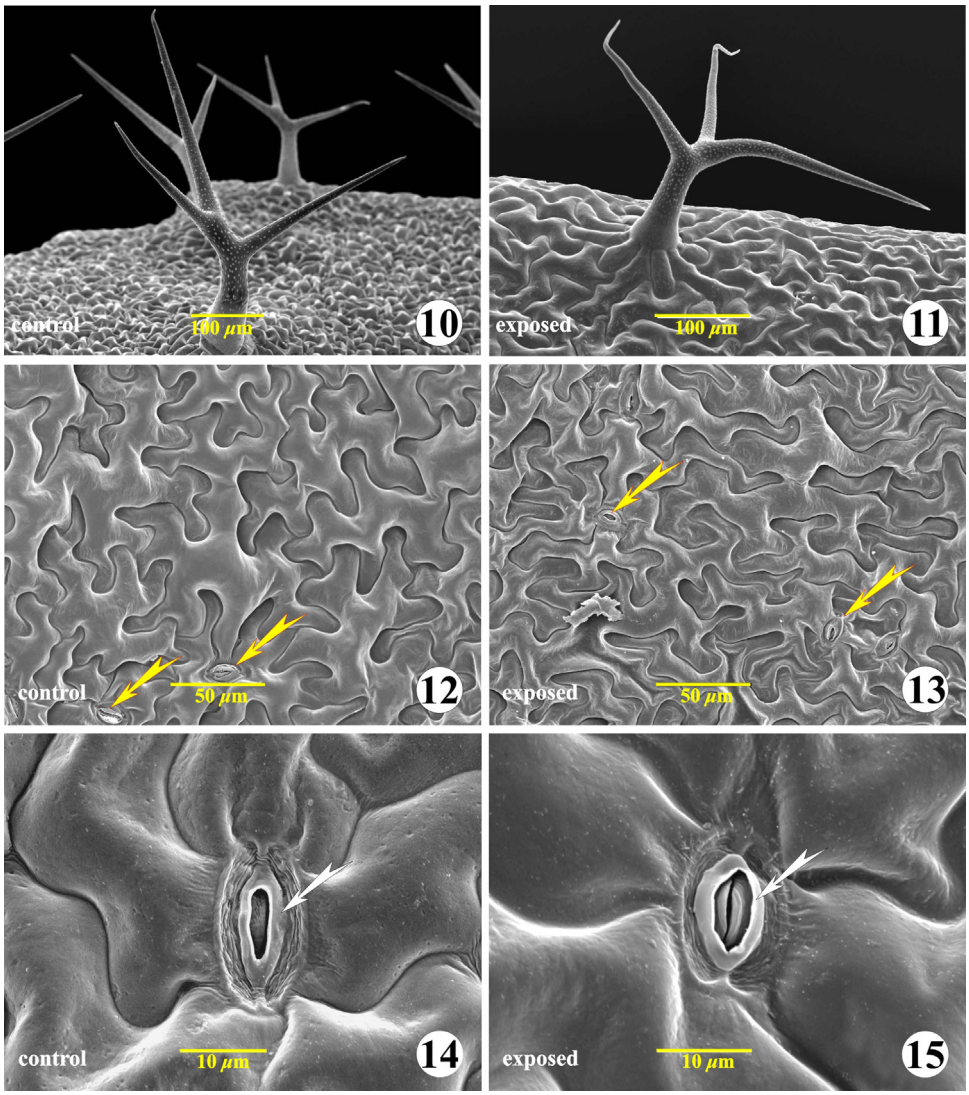
Fig. 9. The photosynthetic pigment content as measured for the control and exposed plants of *A. thaliana* (Col.) in two experiments (mean values, second and third experiment).

4. Discussion

The data presented originate from three experiments with identical set-up but different time length: two weeks for the first experiment, three weeks for the second and four weeks for the last. In each experiment identical conditions were secured for each one of the two Faraday cages with the addition of a continuously transmitting DECT base unit, in one of them. Eventually we investigated the effect of the certain radiation at three stages of the *A. thaliana* (Col.) life span.

In each one of the three experiments we observed differences between the control plants and those grown in the cage with the DECT base unit radiation at an electrical field intensity of 2072 V/m which is far below the ICNIRP (1998) guidelines ensuring non-thermal effects. These differences concern plant development and terminal crop (the biomass of the above the ground parts plus the biomass of the roots), development of the leaves, structure and number of chloroplasts and photosynthetic pigment content (mainly carotenes and chlorophyll–a).

Concerning plant growth, our observations indicate a slowdown in exposed plants since the biomass of this group seems, always, significantly lower. Root growth proves to be readily affected, since roots appear to be retarded more than the above-ground part. Roots growing within each pot intervene with each other and, being so fragile, it is difficult to separate them. Therefore, the total weight of the roots was measured. It is reported that roots seem to be affected by microwave (915 MHz) radiation in concern to the induction of a significant increase of micronuclei after exposure, ranging from a 2.3–fold increase above the sham value, at the lowest specific absorbance rate (SAR) level and up to a 7–fold increase at the highest SAR (Gustavino et al., 2015). Moreover, the number of nodules developed both in *Pisum sativum* and *Trigonella foenum–graecum* is reported to increase with the increase in the radiation exposure (Sharma and Parihar, 2014), yet no data is available for the effect of this radiation on root growth and biomass. Commenting on our data we suggest that roots are readily affected at the first stages of the plant growth (Table 1) and gain their growth rate later; although the biomass at the end of each experiment is still inferior to that of the control plants.

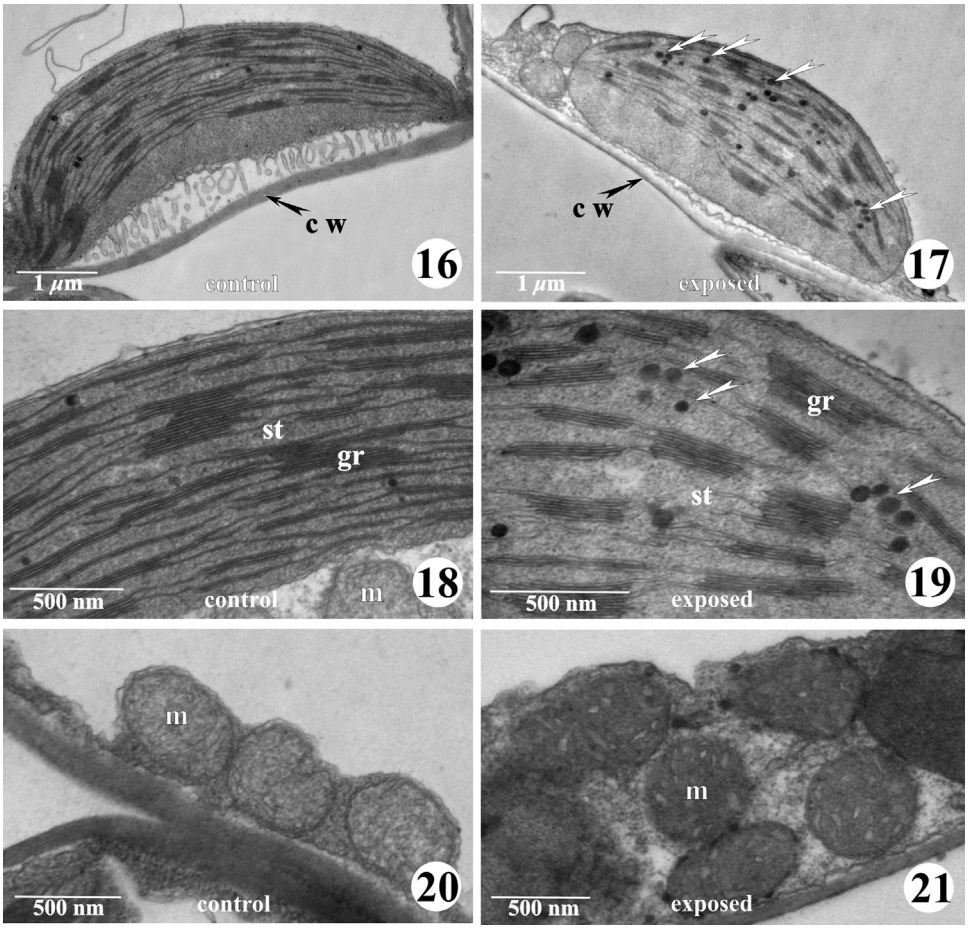


Figs 10–15. Scanning electron micrographs from normal (left) and exposed (right) plants. 10. Unicellular, branched hair on the upper surface of a normal leaf. 11. Unicellular branched hair from the upper epidermis of an exposed leaf. Trichomes on the exposed leaves are far inferior in number. 12. The lower epidermis of a normal leaf. No trichomes appear. Note the large lobed cells and the stomata (arrows), 13. The lower epidermis of an exposed leaf. Cells appear slightly deformed. Stomatal density is uniform in both leaf types. 14. A closed stoma on the lower epidermis of a normal leaf. 15. A partly opened stoma on the lower epidermis of an exposed leaf. White arrows, in Figs. 14 and 15, point at the stomatal edge.

In particular, the control/treated ratio for dry weight, from 2.25 (above the ground part) and 4.09 (root) for the 2 weeks experiment, drops to 1.53 and 1.68 respectively for the 3 weeks experiment and further more to 1.17 and 1.5 for the four weeks experiment, when the life cycle of *A. thaliana* (Col.) is virtually completed. That means that growth is retarded at the early stages of plant life and, as time goes by, the inhibiting effect is gradually minimized.

Scanning electron micrographs reveal no deformations on the surface of the leaves but only a drastic reduction in the number of the unicellular branched hair of the exposed leaves. To the best of our knowledge this effect is reported for the first time and it is not easy to be evaluated. Since DECT radiation is present all over within the cage it seems that developing leaves are exposed from the beginning of their emergence over the soil surface and a programmed-to-be-formed hair (trichoblast) receives the strong dose of electromagnetic radiation which, eventually, inhibits growth.

Exposed leaves appear to be half as thick as the control ones possibly for the reasons mentioned above, while the number of their chloroplasts in the mesophyll cells seems to be drastically reduced. This probably has to do with the stressing conditions imposed by the 1800 MHz radiation which in other systems induces ROS levels increase (Manta et al., 2014). In general, plants experiencing stress exhibit a reduction in the number of their chloroplasts and their pigment content (Zhang et al., 2016). They also present a less elaborated or severely disturbed fretwork in their chloroplasts (Psaras and Christodoulakis, 1987; Christodoulakis and Fasseas, 1990) and an increase in the number and size of plastoglobuli (Lianopoulou et al., 2015; Shao et al., 2016). Our observations on mesophyll cells, with transmission electron microscope, reveal that chloroplasts from the cells of the exposed leaves, although they do not exhibit even the slightest destruction of their membrane system, possess a less elaborated fretwork (stroma thylakoids) implying less space for photosystem I, strong reduction of the pigment content – which is in agreement with current literature (Lianopoulou et al., 2015; Shao et al., 2016) – and, consequently, less cyclic photophosphorylation. This means that exposed leaves are less productive, due to the lower rates of photosynthesis, thus the total yield (biomass) of the exposed plants appears inferior. The lower photosynthetic pigment content and the weight of biomass of the exposed plants in



Figs 16–21. Transmission electron micrographs from the leaves of normal (left) and exposed (right) plants. 16. Chloroplast from a leaf of the control group. 17. Chloroplast from a leaf of the exposed group leaf (white arrows point at plastoglobuli). 18. Detail of a chloroplast from a leaf of the control group. 19. Detail of a chloroplast from a leaf of the control group (white arrows point at plastoglobuli). 20. Detail from a leaf of the control group exhibiting the cell wall and mitochondria. 21. Detail from a leaf of the exposed group exhibiting the cell wall and mitochondria. cw → cell wall, st → stroma, gr → granum, m → mitochondrion.

our experiments confirm the above statement. Furthermore chloroplasts from the photosynthetic tissue of the exposed plants possess numerous plastoglobuli which are formed in their stroma. The presence of these electron dense structures is one of the early indications for an environmental stress (Wellburn et al., 1972; Psaras and Christodoulakis, 1987; Christodoulakis and Fasseas, 1990; Lianopoulou et al., 2015; Shao et al., 2016). Mitochondria and microsomes which are also very susceptible to environmental stress (Wellburn et al., 1972; Psaras and Christodoulakis, 1987) seem to retain their original structure in the cells of the exposed leaves, an indication that the radiation stress conditions are not severe enough to disturb these sub–cellular structures.

5. Conclusion

Long term exposure to non ionizing radiation at the microwave band, has an effect that can be considered as rather serious for this model plant. The reduction in the number of chloroplasts as well as the decrease of stroma thylakoids and the photosynthetic pigments, result in a weak photosynthetic potential and a consequent reduction of the primary productivity.

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