Flora 234 (2017) 173–186

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Original Research

The aftermath of long-term exposure to non-ionizing radiation on laboratory cultivated pine plants (*Pinus halepensis* M.)

Aikaterina L. Stefi[a], Lukas H. Margaritis[b], Nikolaos S. Christodoulakis[a,*]

[a] Department of Botany, Faculty of Biology, National and Kapodistrian University of Athens, Ilisia, Athens, 15701, Hellas, Greece
[b] Department of Cell Biology & Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, Ilisia, Athens, 15701, Hellas, Greece

ARTICLE INFO

Article history:
Received 27 May 2017
Received in revised form 17 July 2017
Accepted 26 July 2017
Edited by Alessio Papini
Available online 1 August 2017

Keywords:
Leaf anatomy
Chloroplast membranes
Root anatomy
Stem anatomy
Chloroplast deformations

ABSTRACT

Sprouts of *Pinus halepensis* were incubated and cultured in the laboratory under controlled conditions to investigate their response to a long-term exposure to continuous, non-ionizing radiation emitted from the base unit of a cordless DECT system. Exposed plants, compared to their control counterparts, seem to be affected since they exhibit lower sprouting potential, minor fresh weight and biomass for both the above ground part and the root, reduction of their photosynthetic pigments and significantly increased ROS levels. Cotyledon, juvenile leaf, primary shoot and root structure seem similar in both control and exposed plants. What seems to be affected is the structure of chloroplasts in the exposed leaves. Many cells of the exposed leaves possess severely deformed chloroplasts with dilated or destructed thylakoid membranes although disruption of chloroplast envelopes was not observed.

1. Introduction

Much consideration has been given and an extensive argument persists on the biological effects of electromagnetic (EM) radiation. Visible light, UV, LASER, X-rays and Gamma rays are EM radiations differing in frequency and, consequently, in energy (Kovács and Keresztes, 2002; Esnault et al., 2010; Abbas et al., 2017; Bibi et al., 2017). Ionizing radiation imposes living organisms to a series of alterations, usually leading to a biological injury (Sax, 1942; Jacobs, 1998; Gulston et al., 2002; Georgakilas et al., 2004; Prasad et al., 2004; Hardell and Carlberg, 2009; Yang et al., 2013). For instance, UV radiation seems to affect plant life in spite of the fact that plants, having the ability to exploit sunlight, do have some means of protecting the living cells from the radiation–induced damages of DNA (Hollósy, 2002). Gamma radiation, as well, 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).

Considering that Earth's natural radiofrequency environment has remained more or less unaltered within the lifespan of modern trees since before 1800, the major components of this environment were the radio emissions from space (galactic noise), the electric discharges within the atmosphere (atmospheric noise), and the small Radio Frequency (RF) component from the sun (Haggerty, 2010). Therefore, we may assume that plants evolved learning to use these environmental signals, along with visible light in order to regulate their periodic functions. That means they may be sensitive to man-made RF fields (Haggerty, 2010).

Since the second half of the 20th century, wireless telephones and, recently, mobile phones turned to be the most common form of communication. This means that organisms living in the "civilized" world thrive within a "cloud" of radiations, mostly non-ionizing. 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). Although plants constitute an outstanding model to study the effect of High Frequency non-ionizing Electromagnetic Fields (HF-EMF) since their architecture (high surface area to volume ratio) optimizes their interaction with the environment (Vian et al., 2016), limited concern was given to plant reactions (Ledoigt, 2006; Roux et al., 2006; Pietruszewski et al., 2007; Haggerty, 2010; Kumar et al., 2015). Recently some data became available, on the biomass production, leaf anatomy and tissue organization, overall, for two widely used dicotyledons – the delicate, short-living, *Arabidopsis thaliana* (L.) Heynh, (Stefi et al., 2016) as a major experimental model plant and *Gossypium hirsutum* L. (upland cotton) as

* Corresponding author.
E-mail address: nchristo@biol.uoa.gr (N.S. Christodoulakis).

http://dx.doi.org/10.1016/j.flora.2017.07.016
0367-2530/

a widely cultivated crop plant of great economic importance (Stefi et al., 2017a) after their long term exposure to non-ionizing, DECT emitted radiation. *Zea mays* was also investigated lately and deformations of the thylakoid membranes in the agranal chloroplasts of the bundle sheath cells (C_4 chloroplasts) was reported, after long term exposure of laboratory cultivated plants to non-ionizing radiation (Stefi et al., 2017b).

The tree commonly known as Aleppo pine (*Pinus halepensis*), is a species native to the Mediterranean region. It grows wild from Morocco to East, in Greece, all over Malta and northern Tunisia and has a prominent population in Syria, Lebanon, southern Turkey, Jordan, Israel and the Palestinian territories. In Israel, it is called Jerusalem pine while, in Greece, the resin of this pine tree, amongst other uses, is used to flavor a Greek type of traditional wine named retsina. Having in mind that most lowlands in Greece are covered with pine trees, mainly the species *Pinus halepensis* (Mill.), and considering conifers (division: Pinophyta) as a plant group with major contribution to the global primary production, particularly in northern areas, we pondered on the impact of the EM radiation on the pine trees and we attempted, primarily, a literature search with interesting results.

Pine trees have previously been investigated for their response to various stressing factors. It is reported, for *P. halepensis*, that although Rubisco activity is reduced by 30% in the presence of ozone, no significant effect is noticed when plants were subjected to water stress (Pelloux et al., 2001). More data is given for *P. halepensis*, concerning the effect of air pollution on the nitrogen cycle (Wellburn et al., 1996), water stress on morphology, physiology and field performance (Royo et al., 2001), gamma-radiation on shoot viability through mitotic inhibition of the apical and subapical meristems (Donini, 1967) while serious effects of gamma (Brandenburg et al., 1962) and UV-B radiation (Laakso et al., 2000) were reported for other pine species. An interesting investigation reports the severe histological changes in the annual rings, the late wood, the number of the vertical resin ducts and the radial rays for two pine species in the proximal zone around the Chernobyl power plant (Skuterud et al., 1994).

Considering all the above we decided to probe the impact of the considered "innocent", continuously emitted, non–ionizing, DECT EM radiation, on the young *P. halepensis* plants, investigating the effects on the cotyledons, leaves, shoots and roots.

2. Materials and methods

2.1. Plant material and exposure setup

Mature, new cones of *Pinus halepensis* Mill., were collected in early September 2016, from the "Ioulia and Alexandros N. Diomidis" Botanical Garden, West of Athens metropolitan area (38°00′34″ N, 23°38′43″ E, 309 m elevation). The cones were incubated at 120 °C for 2 min and then left for five days exposed to sunlight, during the day, for their scales to open. The seeds collected after scale opening were placed in Petri dishes for imbibition. Petri dishes were incubated in a ventilated, adjustable temperature P-Selecta incubator (Model No. 2000238–Barcelona, Spain) where they remained, for ten days, at 20 °C (70% humidity) until seeds sprouted. A build-in light source (Philips CorePro LED bulb, 11.5W = 75W, at 2700 K, 105 mA), adjusted to a 16/8 light/dark cycle, provided 2500 Lux radiation (Photosynthetically Active Radiation = 60 μmol m^{-2} s^{-1}) at the surface of the Petri dishes. After about twelve days, the eight cotyledons emerged and most seeds had grown to young seedlings (2n sporophytes). Selected plantlets were transferred and sown in 80 × 80 mm, 400 mL pots filled with wet perlite (amorphous volcanic glass formed by the hydration of obsidian), at pH 6,0. One young plant was sown in each pot (Fig. 1). Twelve (12) pots were placed to fit in a transparent, rectangular 32 × 20 cm basin, where 20 mL of hydroponic nutrient solution (Hoagland) was added. Then the pot-containing basins were accommodated one in each of the two Faraday cages (40 × 40 × 25 cm, covered with 0.8 mm mesh – 0.1 mm stainless steel wire) (Fig. 2). After their construction, these cages were thoroughly tested for their ability to prevent any leakage of radiation emitted from within, to the environment. The light source (Philips CorePro LED bulb, 11.5 W = 75 W, at 2700 K, 105 mA), mounted on the ceiling of each cage, produced 2500 lux radiation (Photosynthetically Active Radiation = 60 μmol m^{-2} s^{-1}) at the surface of the pots.

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

A ventilated, adjustable temperature incubator (P-Selecta) was used and the two cages were accommodated in it, side by side. The plantlets remained there for 50 days, at 20 °C. The cage on the right hosted the base unit of a Digital Enhanced Cordless Technology (DECT) telephone (General, Model 123) (Fig. 2). The DECT base unit was positioned in the cage in the middle of the two groups of pots (Fig. 2) and remained there in a 24 h a day, 7 days a week, pulsed transmission mode, at 1882 MHz, as described by Margaritis et al. (2014), while the light/dark programme of the chamber was adjusted to a 16/8 cycle (Stefi et al., 2016, 2017a, 2017b). Pots were watered every 5 days with 100 mL tap water. Incubator temperature was set at 20 °C since this was reported to be the proper temperature for optimal germination and growth rate of *P. halepensis* (Thanos and Skordilis, 1987).

Radiation was measured within each one of the two cages while the DECT unit was in transmission mode within the right cage (Fig. 2). A NARDA SRM3000 (Germany) spectrum analyzer was used for these measurements. 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. For cross-checking the above measurements, a broadband field meter was used (TES-92, 50 MHz – 3.5 GHz, Electromagnetic radiation detector – TES Electrical Electronic Corp. Taipei, Taiwan, R.O.C.). Although this instrument is of lower precision, radiation was, once more, measured at the value of 490.1 mV/m within the control cage (Fig. 2–left cage) while it reached the value of 27.46 V/m (27.460 mV/m, at 1882 MHz) (55 fold higher) in the cage of the exposed plants (Fig. 2–right cage).

2.2. Microscopy

At the end of the experimental period, the pots were removed from the cages and smashed in water to acquire the plants which were washed to remove any remnants of the culturing substrate from the roots and placed on a filter paper (Fig. 3). The fresh weight of the plants in each group was measured before the plants were incubated for drying, at 60 °C, for three days. Then the dry weight (biomass) was measured. In all plants the fresh weight and the dry biomass were weighed for their above ground part and their root system. A small part from the centre of one cotyledon from each plant (yellow bar A in Fig. 4), one leaf from each plant, a part from the middle of the stem (yellow bar B in Fig. 4) and a part of the root, 5 cm bellow the shoot (yellow bar C in Fig. 4) from each plant, were removed in random, sectioned in to small (2 mm length) pieces and fixed in phosphate buffered 3% glutaraldehyde (pH 6.8) at 0 °C for 2 h. Some of the pieces, from each organ, were dehydrated in