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Plant Sensitivity to Low Intensity 105 GHz Electromagnetic Radiation

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Exposing seedlings of the flax, *Linum usitatissimum* L., to a variety of weak environmental stresses followed by a 2 day calcium deprivation, triggers the common response of production of epidermal meristems (actively dividing groups of cells) in the hypocotyl, which is the part of the stem between the root and the cotyledons (the pre-existing leaves in the embryo). This production reaches a plateau of 10–20 meristems after a month in the case of mechanical stimulation and cold shock. Recently, we have shown that radiation from a global system for mobile communication (GSM) telephone also triggers production of meristems with a plateau of around six meristems. Here, we show that a single 2 h exposure to radiation emitted at 105 GHz at non-thermal levels by a Gunn oscillator induces meristem production with kinetics similar to that induced by weak environmental stimuli and radiation from GSM telephone. Bioelectromagnetics 00:1–5, 2003. © 2003 Wiley-Liss, Inc.

Key words: environmental stress; microwaves; flax; meristem; calcium

INTRODUCTION

Plants are exposed to a variety of natural abiotic and biotic stresses, such as drought, rain, thermal stresses, wind, mechanical contact, pricking by insects, wounds inflicted by phytophages, and infection by pathogens. These stresses generate intracellular signals [Braam and Davis, 1990; Braam et al., 1996; Jonak et al., 1996; Mizoguchi et al., 1997; Meskiene and Hirt, 2000], such as calcium transients [Knight et al., 1991, 1992, 1996; Takahashi et al., 1992; Malhó et al., 1998; Plieth et al., 1998; Mithöfer et al., 1999], that lead to modifications of growth rate and/or morphogenesis of the whole plant [Adams, 1924; Jaffe, 1985; Thellier et al., 2000].

Our group has developed an experimental system that clearly reveals the effects of environmental stimuli [Verdus et al., 1996, 1997]. In this system, week-old seedlings of the flax *Linum usitatissimum* L. var Ariane are subjected to a temporary (typically 2 days) calcium deprivation by replacing the complete growth medium with a calcium-depleted medium. After this depletion the plants are again grown on the complete medium. This results in the production of epidermal meristems in the hypocotyls, typically 10–20 meristems after a month, provided the seedlings have previously undergone an exposure to different stresses or stimuli, such as drought or touch, respectively. In plants, meristems are small groups of actively dividing cells that after differentiation ultimately produce stem or root tissues.

The hypocotyl is the part of the stem located between the root and the cotyledons (pre-existing leaves in the embryo). In control experiments designed to minimize these stresses, the mean number of meristems produced per plant is 0 or 1 after subjecting the plants to calcium deprivation.

Recent experiments have shown that exposure to radiation emitted from a global system for mobile communication (GSM) telephone followed by calcium deprivation, leads to meristem production in our flax system [Tafforeau et al., 2002]. In their natural environment, plants are also exposed to radiation at a large range of other frequencies. Electromagnetic radiation in the 100 GHz range is already widely used in a variety of communications systems and environmental sensors, such as telecommunications links and earth resources

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monitoring applications; and it is therefore important to explore the effects of exposure of biological systems to radiation at these frequencies. To investigate the possibility that plants may be affected by exposure to a frequency in this range, we used the sensitive experimental system described above. We report below how irradiation of flax seedlings with a Gunn oscillator at 105 GHz induced meristem production comparable to that induced in seedlings subjected to mechanical stimulation, cold shock, and other environmental stresses or stimuli [Verdus et al., 1997; Tafforeau et al., 2002].

MATERIAL AND METHODS

Plant Growth and Calcium Deprivation

Plants were grown in a culture room at 22 ± 1 °C under continuous artificial light (6.4 W/m^2 irradiance), except for a 3 day period of germination in the dark. The culture medium, slightly modified from the standard Homès medium, contained the macronutrients (mM) KNO_3 6.92; $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 2.33; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.62; and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 2.18 and the micronutrients (μM) $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 6.86; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 1.0; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 1.04; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 0.03; $\text{Fe}^{3+}\text{EDTA}$ 51.0; and H_3BO_3 27.33. Each batch of approximately 150 flax seeds was grown in its own culture box. Polystyrene culture boxes (Sercobox, Polylabo) and polypropylene meshes (Scrynel, Polylabo) were used.

Two simultaneous replicates in each of two sequential independent experiments were carried out. Seeds were placed on polypropylene meshes fitted on the top of each box, and the nutrient medium was brought into contact with them (days 1 and 2) to allow germination. The seedlings were then left to grow for a further 3 days in situ. On day 6, separate batches of seedlings were exposed to a 2 h irradiation using a Gunn oscillator (see below).

After each irradiation, several batches were subjected to a temporary calcium deprivation to induce the formation of meristems. To perform the temporary calcium depletion, the complete medium was substituted during days 7 and 8 by a calcium-deprived medium. The seedlings were then left to grow on the complete medium until day 29. In the calcium-deprived medium, calcium was replaced by potassium, and the concentrations of nitrate, sulfate, and phosphate were kept to the same value as in the complete medium. The variations in the total osmolarity and ionic strength were thus negligible. The medium was renewed every 2 days throughout the experiment.

To count meristems, 10 seedlings were taken at random from each of the batches (on days 9, 11, 12, 13, 15, 18, 20, 22, 25, 27, 29) and immediately plunged in

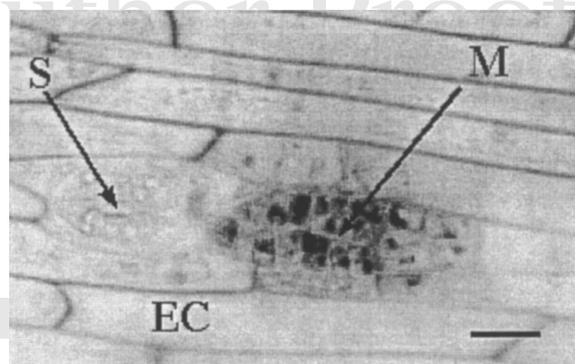


Fig. 1. Optical view of a newly developed meristem in the epidermis of the flax hypocotyl. A meristem (M) is clearly distinguished from the normal epidermal cells (EC) or from the stomata (S). Bar = 30 μm .

50% (v/v) aqueous ethanol at room temperature for 24 h or longer, until they became transparent enough to allow counting of the epidermal meristems using a Leica light microscope (model DMRB). Only meristems comprising at least four newly divided cells were counted (Fig. 1).

Plant Irradiation

All culture boxes (control and experimental) were placed in the same culture room. For irradiation (on day 6) the corresponding boxes were carefully carried the 10 m distance to another culture room, where the temperature and light intensity were the same, where the Gunn oscillator was located; the radiation from the oscillator reaching the first room was thus negligible. After the 2 h irradiation (see details below), the culture boxes were carefully taken back to the first culture room. We checked in four replicates that carrying the boxes from one room to the other did not cause any significant production of meristem after calcium deprivation in the absence of irradiation (data not shown). Other slight disturbances to the seedlings, such as those induced by the sampling and the renewal of the growth medium, were also without effect.

Seedling irradiation was carried out at 105 GHz for 2 h using a 50 mW Gunn oscillator mounted above them (Fig. 2). The output power was monitored using a Golay cell millimeter wave detector. A Golay cell is a sensitive room temperature detector that operates between wavelengths of ~ 3 and 0.1 mm. It is comprised of a gas chamber and a parallel planar capacitor consisting of a thin diaphragm. Millimeter wave radiation heats the gas in the chamber, and the deflection of the diaphragm induced by the expansion of the gas is sensed by a photo-optic sensor, such that the voltage output is proportional to the power detected. The detection area

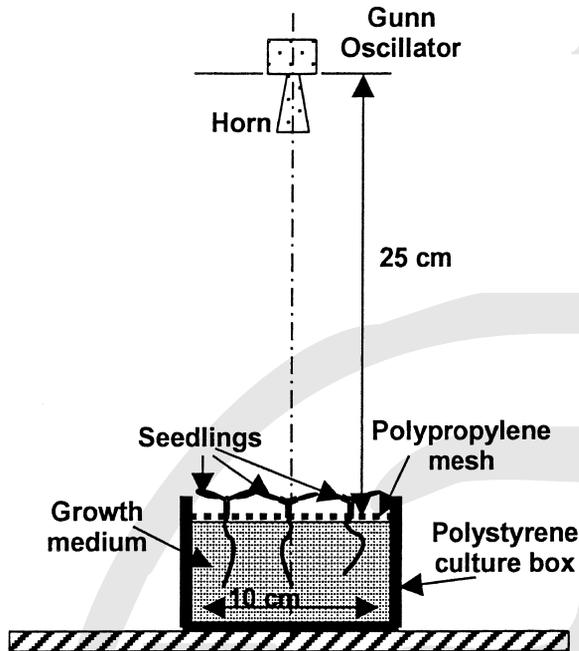


Fig. 2. Schematic representation of the experimental apparatus used for the irradiation of flax seedlings. For clarity only three seedlings are represented here; in the actual experiments, approximately 150 seedlings were grown on the polypropylene mesh.

of the Golay cell is 1.22 cm^2 . The output angle of the 105 GHz beam from the Gunn oscillator was 12° , leading to an estimated mean power density of the order of 10 W/m^2 at the base of the hypocotyls. The absolute power level of 105 GHz radiation incident at the sample was calibrated against an absolute power meter which has an accuracy of $\pm 10\%$ (<http://josephson.terahertz.co.uk/TKI/tkins.html>). This power meter was placed at the same distance from the end of the Gunn oscillator feed horn as the sample (25 cm). The power distribution across the area occupied by the sample was measured using the Golay detector and found to vary by less than $\pm 25\%$ from the center to the periphery of the irradiated area with no other hot spots discernable.

RESULTS

Seedlings that, on day 6, were exposed to radiation from the Gunn oscillator produced an average of seven meristems 3 weeks (day 29) after the end of the calcium deprivation (Fig. 3). The controls performed were: (1) irradiation without the calcium deprivation step, (2) no irradiation but inclusion of the calcium deprivation step, and (3) no irradiation and no calcium deprivation. Controls 1 (not shown) and 2 (Fig. 3) gave an average of less than 0.7 meristems on day 29, while control 3 gave none (data not shown). There is no appreciable dif-

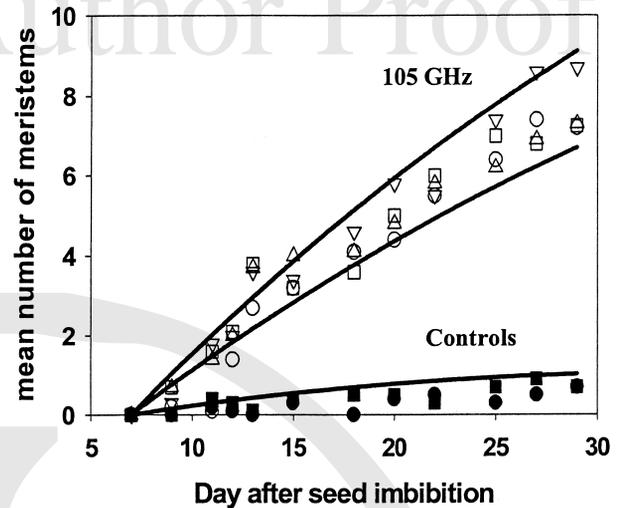


Fig. 3. Mean number of meristems per hypocotyl as a function of time. A total of 1144 seedlings were analyzed. Each point is the mean of 10 measurements. Each separate experiment is represented by a different symbol. The lines around these points are arbitrarily drawn for visual help to show the difference between irradiated and control seedlings. Irradiation by the Gunn oscillator, open symbols; controls (no irradiation but calcium deprivation), filled symbols.

ference between the results of giving the same treatment in separate experiments. By contrast there is a clear-cut difference between the results of a 2 h irradiation and the controls, as shown Figure 3.

A microwave dissipates as heat a fraction $(1 - e^{-d/\delta})$ of its energy when it penetrates to a depth d into a material. δ is a characteristic length, the penetration depth, given approximately by the expression $\delta = c(\epsilon_r')^{1/2} / (2\pi\nu\epsilon_r'')$ where c is the speed of light in the vacuum, ν the frequency of the wave, ϵ_r' the real part of the complex relative permittivity of the material, and ϵ_r'' its imaginary part corresponding to the dielectric loss factor (see, e.g., <http://www.sbu.ac.uk/water/microwave.html>). For a wave at 105 GHz, $\delta = 0.45(\epsilon_r')^{1/2} / (\epsilon_r'')$ mm. For pure water or moderately concentrated salt solutions at 20°C , $\epsilon_r' = 8$ and $\epsilon_r'' = 12$ (e.g., www.sbu.ac.uk/water/microwave.html), and hence $\delta = 0.1$ mm. Therefore, a wave that has penetrated to a depth 4δ , that is, 0.4 mm, into water has dissipated 98% of its energy; we assume the same holds true for plant tissues. This means that a wave at 105 GHz penetrates only superficially into the growth medium, whilst it penetrates to the core of the hypocotyl in the case of a wave with an incidence normal to the surface into which it penetrates. The polystyrene of the culture box and the polypropylene of the meshes are transparent at this frequency.

To estimate the increase in temperature, ΔT , of the thin layer of growth medium within which the wave energy is dissipated, assumed to be a slab of water with planar faces maintained at constant temperature, we have used the standard equation $\Delta T = A_0 l^2 / (2\kappa)$ where A_0 is the power dissipated per unit volume (W/m^3), l the thickness (m) of the slab, and κ the thermal conductivity (W/mK) [Carslaw and Jaeger, 1971]. This relation may be rewritten $\Delta T = 2 \cdot PD \cdot \delta / \kappa$ where PD is the mean power density (W/m^2) existing in the sample and l is assumed to be equal to 4δ . The Gunn oscillator delivered a mean power density of a maximum of 10 W/m^2 at the level of the meshes, which gives a calculated value of $\Delta T = 0.013 \text{ K}$, i.e., a negligible temperature increase. To eliminate the possibility that the plants themselves were heated, a similar calculation can be performed by treating the seedlings as roughly cylindrical [Carslaw and Jaeger, 1971]. The maximum temperature increase is then given by the same formula as for the water slab, $\Delta T = 2 \cdot PD \cdot \delta / \kappa$, because we have chosen 4δ as the radius of the cylinder; 0.4 mm is indeed close to the radius of the hypocotyl. Therefore, the temperature increase is also negligible within the plants.

To confirm that this power level did not result in a significant local heating of plant tissue, four tests were conducted in a temperature-stable environment, that is, controlled to better than $0.1 \text{ }^\circ\text{C}$, on water droplets that were 2 mm in diameter. The droplet was supported on a thermistor temperature sensor housed in a thermally insulated box and was compared against a similar water droplet as a control sample housed in the same chamber, but not irradiated by the 105 GHz radiation. The test droplet was then irradiated with 105 GHz radiation for 30 min . It did not prove possible to deliver a higher power to the test drop, because the diameter (beam waist) of a focused beam of millimeter wave is typically a few mm diameter; this is the smallest area into which the radiation can be focused. Based on the measured beam waist size, and the geometrical area of the water drop, we estimate that $5 \pm 0.5 \text{ mW}$ of 105 GHz radiation were delivered to the water droplet at a power density of 400 W/m^2 . No rise in temperature above the detection limit of $0.1 \text{ }^\circ\text{C}$ was observed either in the presence or absence of the water droplet; this result is consistent with the above calculations. It should be noted that this power density is much higher than the power density of irradiation delivered to the plants, and therefore this test supports the conclusion that, as expected, no significant temperature raise occurred during their irradiation.

These results show that flax seedlings respond to the combination of a 2 h irradiation at 105 GHz plus a 2 day calcium deprivation in the same way as they

respond to other combinations of a stimulus such as touch, cold shock, wind, or drought plus calcium deprivation, namely, by epidermal meristem production in the hypocotyls [Verdus et al., 1997; Tafforeau et al., 2002].

DISCUSSION AND CONCLUSIONS

To study the long term storage of a variety of environmental signals, we have developed the system of epidermal meristem production in the hypocotyls of flax following a calcium deprivation step. This system is particularly effective for revealing the transduction of a wide range of low intensity stimuli by seedlings [Verdus et al., 1997]. These stimuli include physical stresses such as cold shock, drought, and mechanical stresses. Recently, we found that a 2 h exposure to radiation from a GSM telephone also induces epidermal meristem production in the flax system [Tafforeau et al., 2002].

Here, we have investigated what happens to flax seedlings in our system when they are exposed to radiation at a very different frequency emitted by a very different device. They respond to non-thermal levels of radiation at 105 GHz from a Gunn oscillator by a production of epidermal meristems in a way that is similar to their response to other physical stimuli. The downstream part of the signal transduction mechanism for a wide variety of stimuli and stresses is thus clearly the same, insofar as there is a common response of meristem production; indeed, the response to exposure to 105 GHz reveals the same calcium dependence and the same kinetics as the response to other physical stimuli including radiation from a GSM telephone. The question, however, might be raised as to whether the radiation from the telephone and the Gunn oscillator affect the same specific target. We cannot exclude the possibility that different, specific, biological processes are perturbed separately and that these perturbations generate a common stress response.

It should be noted that a stress protein is synthesized in a very different system that is based on exposure of the nematode *Caenorhabditis elegans* to non-thermal levels of radiation at a frequency similar to that emitted by a GSM telephone [de Pomerai et al., 2000]. Our findings extend the sensitivity of biological systems to non-thermal levels of radiation to plants. It should be emphasized that whilst meristem production is in itself a significant biological consequence of irradiation at 105 GHz , this is not necessarily an adverse consequence. Other abiotic treatments such as touching also lead to flax seedlings producing meristems after calcium deprivation. These seedlings show no evidence of damage, and their growth rate, buds, and shoots are all normal.

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