Systemic accumulation of bZIP mRNA after Low Amplitude 900 MHz stimulation in plant

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Abstract- We demonstrate in the present work that low amplitude 900 MHz stimulus constitutes a true environmental stimulus because it induces an immediate drop (60%) in bZIP transcription factor mRNA quantity, and a transmission of a traumatic signal. We also show that calmodulin transcripts increase after similar treatment, indicating a possible involvement of calcium metabolism in the response to EMF stimulus.

I. Introduction

In nature, plants are continuously subjected to various kind of stimuli (wind, rain, pathogens attaks, drought, UV light...). Because of their immobility, they cannot escape and must therefore sense these environmental variations and adapt their development accordingdly. In the present days, the growing use of wireless communication devices leads to an important increase in the density of human-generated Electromagnetic Field (EMF) in our immediate environment. This point adresses numerous questions about possible effects on lifeforms, especially human beings. Thus, numerous reports concentrate on these aspects [1], mainly with the 900-1800 MHz signals emitted by cellular phones. Because of medical concerns, investigations were mainly conducted through epidemiologic studies [2]. While being well suited to demonstrate link between causal agent and responses at the population level, we aimed to make a more formal relationship. We therefore set up an experimental design that emphases 3 main points : i) a total control over the EMF stimulation (frequency, amplitude, polarization, duration...) produced within a metallic Mode Stirred Reverberating Chamber (MSRC), ensuring protection from EMF present in the environment ; ii) the choice of plant (tomato, *Lycopersicon esculentum*) as a simplified, immobile and sensitive biological model and iii) a biological marker that can serve as an immediate reporter of metabolic changes (*i.e.* the quantity of messenger RNA of stress-related genes). We have previously showed that plants exposed to 900 Mhz-5 $V.m^{-1}$- 10 min EMF displayed a rapid (5-15 min) and transcient (30-60 min) increase in the quantity of LebZIP1 transcription factor mRNA [3]. In the present report, we describe that similar but weaker increase occurs for calmodulin mRNA after the same HF-EMF treatment. We also demonstrate that exposing a single leaf to EMF radiation (while shielding the rest of the plant) causes the same responses in the distant protected tissues, suggesting the rapid transmission of a traumatic signal within the entire plant.

II. Material And Methods

A. EMF Stimulation

The EMF stimulation is performed using a MSRC that ensures a homogeneous and isotropic electromagnetic field at 900 MHz [3]. Plant are grown for 3 weeks into a EMF-transparent culture chamber, the 4th terminal leaf is immediately harvested after stimulation, and frozen in liquid nitrogen. For shielding experiments, plant are placed into 1 mm tick aluminum EMF-proof container (giving an attenuation factor of 23 dB at 900 MHz) with the first leaf emerging outside (Fig.1). Control plants are placed into the container with no emerging tissues, thus being non-exposed to the EMF radiations.

B. mRNA quantity measurement

RNA is isolated using the TRI-Reagent method (Sigma Chemical), converted to single strand cDNA (Advantage RT-for-PCR, BD Biosciences). This cDNA is used as a matrix to prime quantitative RT-PCR reactions, and the relative mRNA quantity (Qr) is calculated accordingly to the $2^{-\Delta\Delta Ct}$ method [4], relatively to actin messenger RNA quantity, and normalized to the control (non stimulated) reference.

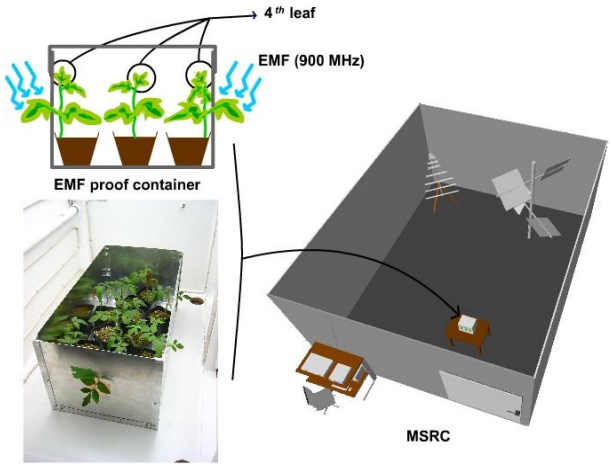

Fig. 1. Experimental set up for shielding experiments. Tomato plants (3-weeks old) are placed either totaly inside an EMF-proof container (control plants) or with the 1st leaf left outside (exposed plants). After exposion to EMF, the 4th (protected) terminal leaf is collected for analysis.

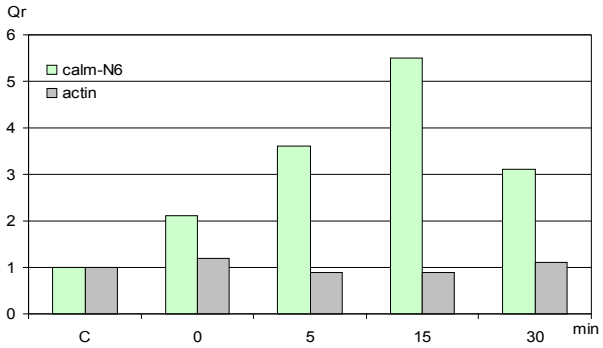

Fig. 2. Variations of the calm-N6 and actin mRNA quantity after EMF exposure of whole plant grown under daylight conditions. Qr: relative mRNA quantity. C: totally shielded plants. 0, 5, 15, 30 : minutes after the stimulation end.

III. Results and Discussion

The calm-N6 [5] mRNA quantity displays in daylight conditions a rapid and transient increase after plant exposition to HF-EMF (Fig.2). This increase is occurring immediately after the stimulation (Fig. 2, lane 0) and is maximum 15 min later (displaying a typical 4 to 5.5-fold increase, Fig. 2, lane 15). In contrast, the quantity of the actin reference messenger RNA remains constant.

This result indicates that calmodulin may be implicated into the plant response to HF-EMF stimulus, as reported for many environmental stresses [6]. It also suggests (while not demonstrates) that calcium metabolism is affected by EMF. Thus, it is likely that a panel of cellular responses (i.e. calcium movements, modulation of phosphorylations, specific transcription factors...) take place rapidly after EMF treatment. In this context, the rapid increase in bZIP mRNA (a stress-related transcription factor [7]) we recently demonstrate can constitutes one of these events.

In darkness, the EMF stimulation caused a decrease

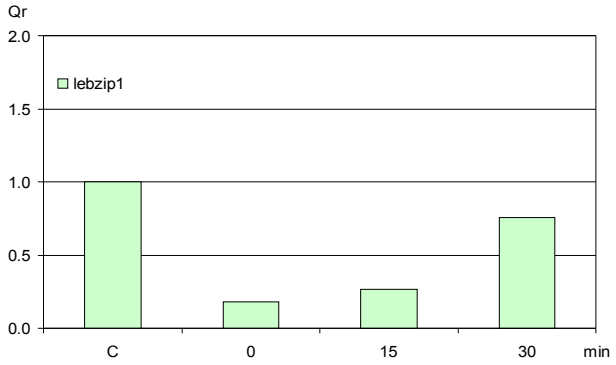

Fig. 3. Variations of the LebZIP1 mRNA quantity after HF-EMF exposure of the whole plant grown under darkness conditions Qr: relative mRNA quantity. C: totally shielded plants.

in the bZIP mRNA quantity (Fig. 3). This result strongly suggests that light constitutes an important parameter regulating the response to EMF stimulus. Similar results were observed for many stress-related gene expression after wound treatements [8,9].

Stress-related responses generally occured systemically (*i.e.* in the entire plant) after a local stimulus. To test if EMF treatment has similar characteristics, we set up the experimental design described in Fig. 1. The first leaf is the only part of the plant exposed to EMF treatment while the analyzed tissue (4th terminal leaf) is protected into the container. This experimental setup implies darkness conditions for plants . Here, as observed for experiments conducted in darkness, the LebZIP1 mRNA quantity displays a rapid and transient decrease (fig. **4**) maximal (60 %) 5 min after the end of the stimulation. The quantity return to an almost normal level after 30 min. During this time, no variation is observed for the actin control mRNA (data not shown). The control plants, totally protected into the container (*i.e.* therefore not exposed to EMF) show no significative variation in the quantity of bZIP or actin transcripts (fig. 5).

The data presented here show that tissues situated at a distance from the site of stimulation (thus not directly stimulated) respond in a similar way than tissues subjected to EMF radiations. This result implies that i) the EMF stimulation constitutes a true environmental stimulus leading toward ii) the transmission of a traumatic signal

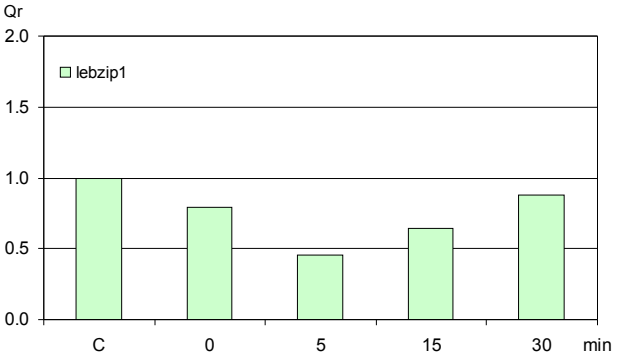

Fig. 4. Shielding experiments. Variations of the LebZIP1 mRNA quantity after exposure of a single leaf (1st leaf exposed, 4th terminal shielded leaf analyzed). Qr : relative mRNA quantity ; C: control (*i.e.* totally shielded plants) ; 0, 5, 15, 30 : minutes after stimulation end . Sample experiment out of 3 similar independant experiments.

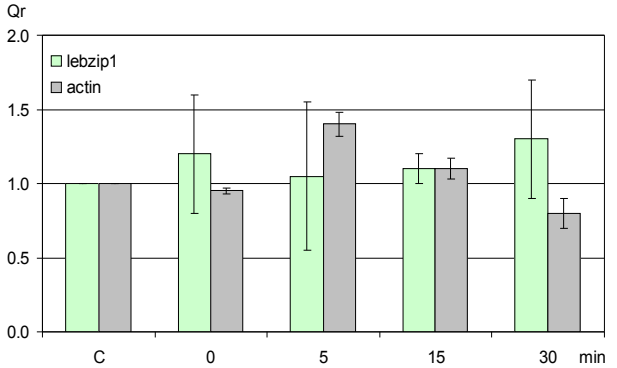

Fig. 5. Shielding experiments control. Variations of the LebZIP1 and actin mRNA quantity ± SD in non EMF-exposed plants (darkness conditions needed). Qr: relative mRNA quantity. C: totally shielded plants.

emitted from the exposed tissue to the entire plant. This message moves rapidly through the plant since molecular responses occured with the same kinetics in exposed or shielded tissues. The exact nature of this message is still hypothetical, while previous works have implicated molecular messengers (ABA, systemin, oligosaccharides...) and/or electric signals (action potential and variation potential) in the long distance signaling in plants [10,11]. Experiments are in progress to decipher if such mechanisms occur after EMF stimulation.

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