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DNA of *Hibiscus sabdariffa* Damaged by Radiation from 900 MHz GSM Antenna

A. O. Oluwajobi, O. A. Falusi, N. A. Zubbair, T. Owoeye, F. Ladejobi, M. C. Dangana, A. Abubakar

Abstract**—**The technology of mobile telephony has positively enhanced human life and reports on the bio safety of the radiation from their antennae have been contradictory, leading to serious litigations and violent protests by residents in several parts of the world. The crave for more information, as requested by WHO in order to resolve this issue, formed the basis for this study on the effect of the radiation from 900 MHz GSM antenna on the DNA of *Hibiscus sabdariffa*. Seeds of *H. sabdariffa* were raised in pots placed in three replicates at 100, 200, 300 and 400 metres from the GSM antennae in three selected test locations and a control where there was no GSM signal. Temperature (˚C) and the relative humidity (%) of study sites were measured for the period of study (24 weeks). Fresh young leaves were harvested from each plant at two, eight and twenty-four weeks after sowing and the DNA extracts were subjected to RAPD-PCR analyses. There were no significant differences between the weather conditions (temperature and relative humidity) in all the study locations. However, significant differences were observed in the intensities of radiations between the control (less than 0.02 V/m) and the test (0.40-1.01 V/m) locations. Data obtained showed that DNA of samples exposed to rays from GSM antenna had various levels of distortions, estimated at 91.67%. Distortions occurred in 58.33% of the samples between 2-8 weeks of exposure while 33.33% of the samples were distorted between 8-24 weeks exposure. Approximately 8.33% of the samples did not show distortions in DNA while 33.33% of the samples had their DNA damaged twice, both at 8 and at 24 weeks of exposure. The study showed that radiation from the 900 MHz GSM antenna is potent enough to cause distortions to DNA of *H. sabdariffa* even within 2-8 weeks of exposure. DNA damage was also independent of the distance from the antenna. These observations would qualify emissions from GSM mast as environmental hazard to the existence of plant biodiversities and all life forms in general. These results will trigger efforts to prevent further erosion of plant genetic resources which have been threatening food security and also the risks posed to living organisms, thereby making our environment very safe for our existence while we still continue to enjoy the benefits of the GSM technology.

Keywords**—**Damage, DNA, GSM antenna, radiation.

A. O. Oluwajobi is with the Department of Science Laboratory Technology, Kwara State Polytechnic, Ilorin, Nigeria (phone: +234-0803-5036-877; e-mail: segunoluwajobi@yahoo.com).

N. A. Zubbair is with the Department of Science Laboratory Technology, Kwara State Polytechnic, Ilorin, Nigeria (nzubbair@gmail.com).

T. Owoeye and F. Ladejobi are with Bioscience Center, International Institute for Tropical Agriculture, Ibadan, Nigeria(e-mail: t.owoeye@cgiar.org, bukkielad@hotmail.com).

O. A. Falusi, M. C. Dangana and A. Abubakar are with Department of Plant Biology, Federal University of Technology, Minna, Nigeria (e-mail: falusiolamide@yahoo.com, danganachata@gmail.com, abuakim2007@yahoo.com).

I. Introduction

THE green Roselle (*Hibiscus sabdariffa* L.) is a widely grown vegetable, eaten in many parts of the world. It belongs to the family Malvaceae and it is cultivated for its calyces and leaves. It is a very hardy plant that can survive under minimal growth conditions. The leaves and calyces are variously used, depending on the locality. They are useful for making jelly, jam, juice, wine, syrup, gelatin, pudding, cake, ice cream and flavours [1]. Roselle plays important roles in local medicinal formulations with claims that it can be used in the treatment of hypertension, pyrexia, liver damage and leukaemia because of its high content of polyphenols [2], [3].

In recent times, there have been agitations on the environmental impact of the increasing mounting of Global System for Mobile (GSM) Telecommunications antennae with contradictory reports on its bio-safety [4]-[11]. There have been several reports of this electromagnetic radiation on plants too, although many of these reports are simulated experiments. References [12] and [13] reported rapid growth of plant root cells on exposure to the weak radio fields while [14], [15], using the Mode Stirred Reverberation Chamber, exposed tomato plants (*Lycopersicon esculentum* Mill) to a 900 MHz, 5 V/m electromagnetic fields reported various stress – related responses typical of an environmental stress response. They concluded that the responses observed in the plants suggest that they are the direct consequence of application of radio – frequency fields and is very similar to their responses to wounds. In a report of preliminary observations of the adverse influence of radio frequency background on Trembling Aspens (*Populus tremuloides* Michx) seedlings, [16] exposed the seedlings of Aspens to radio frequency in open field condition for four months. The control seedlings were placed in a Faraday cage using aluminium window screen. This created an RF signal free environment. The results showed that RF shielded plants produced vigorous growth than the non-shielded plants, producing 60% more leaf area. The exposed plants did not produce fall anthocyanin and there were necrotic lessons in fall senescing leaves. The author concluded that the adverse effects of the radio waves on the growth rate and fall anthocyanin production may be the underlying factor in aspens decline in North America. Reference [17] reported that the radiation from the GSM mast caused a reduction in flower bud abscission in *H. sabdariffa* thus aiding fruit formation in the plant.

Although the electromagnetic radiations from the GSM antenna is generally regarded as weak and harmless, the recent reports on its effects on living organisms in general and especially plants calls for concern in the face of global

challenges on food security and lose of plant genetic diversities. In this light, the research work investigated the impact of the ray emission from the GSM mast on the DNA of the green Roselle.

II. Methodologies

The methods of [17] were adopted for the choice of locations and setting up of the experiments. However, measurements of temperatures (˚C) and relative humidity (%) at each test and control sites were taken weekly and throughout the twenty-four weeks of the experiment using the ordinary mercury thermometer and the Hygrometer respectively. The relative humidity was read out in the Hygrometric table. Field intensities of the radiations in the test and control locations were taken with the Acoustimeter as reported by [18].

Fresh young leaves were collected from the plants in their experimental locations and that in the control. These were placed in appropriately labelled envelopes and the envelopes were packed into waterproof bags in which they are transported to the laboratory in iced blocks for analyses. 1A,1B and 1C labels represent samples from Location A, 100 m at 2 weeks, 8 weeks and 24 weeks respectively while 2A, 2B and 2C being samples from Location A, 200m at 2 weeks, 8 weeks and 24 weeks respectively (Table I). Control A, Control B and Control C are samples from the control location at 2 weeks, 8 weeks and 24 weeks respectively.

Two hundred milligrams of *H. sabdariffa* leaf tissue of each sample was separately ground using liquid nitrogen and was transferred into 1.5 ml Eppendorf tube to which 700 µl of pre-heated plant extraction buffer was added. The tubes were incubated at 65 ^{o}C for 20 minutes with regular mixing by occasionally inverting the tubes to homogenise the samples. The tubes were removed, allowed to cool for 2 minutes and 500 µl of ice-cold 5 M Potassium acetate added and incubated on ice for 20 mins to precipitate protein. They were centrifuged at 12000 rpm for 10 mins and the supernatants were transferred into freshly labelled tubes. 700 µl of Chloroform: Isoamyalcohol (24:1) was added and mixed gently to further precipitate protein and lipids. These were also centrifuge at 12000 rpm for 10 mins and the supernatants transferred into new tubes. 500 µl of ice-cold isopropanol was added, mixed gently and incubated in -80 ^{o}C for 15mins to precipitate the DNA. They were centrifuge at 12000 rpm for 10 mins and the supernatants were decanted until the last drop leaving the DNA pellet at the bottom of the tube. 100 µl of 70 % ethanol was added to wash the DNA pellet. This was spin down and the pellet air dried until no trace of ethanol was found. 60 µl of ultra pure water was added to re-suspend the DNA to which 2 µl of RNase was added. This was incubated in 37 ^{o}C for 30-40 minutes. The DNA quality was checked on 0.8 % Agarose and Nanodrop Spectrophotometer ND-1000. The DNAs were stored at -20 ^{o}C.

The total volume of the PCR reaction was 25 µl, which contained 2.0 µl of template DNA, 2.5 µl of 10X buffer, 1.2 µl 50 mM $MgCl_2$, 1 µl of DMSO, 2.0 µl of 2.5 mM dNTPs, 0.2 µl of Taq polymerase (Bioline), and 10 µl of RAPD primer. The PCR cycle was carried out with 9700 Applied Biosystems Thermal Cycler with the initial denaturation at 94^{o}C for 3 minutes followed by 45 cycles of 94 ^{o}C for 20 seconds, 37 ^{o}C annealing for 40 seconds and 72 ^{o}C for 1 min followed by 72 ^{o}C for a 7 min extension and soak temperature at 10 °C forever. The product was stored at 4 ^{o}C and loaded on 2 % Agarose gel, stained with Ethydium Bromide and it was run at 80 Volts for 4 hrs. The molecular fragments were estimated using 50 base pairs (bp) PCR markers. Photographs of the probes were taken. The data obtained were subjected to analyses using Darwin 5.0 software. Linkage analyses (factorial analysis, hierarchical clustering trees) were done using Unweighted Pair Group Method using Average (UPGMA).

III. Results

The weather data showed no significant differences between the measurements of temperatures and relative humidity in the control and the test locations (Table II). However, radiations intensities between the control (< 0.02 V/m) and the test (0.40-1.01 V/m) locations are significantly different (Table III).

Out of twenty RAPD markers tested, only 9 amplified with a total of 34 alleles and an average amplification of 55.3%. The results showed that DNA samples from the control experiment remained virtually intact from 2 weeks to 24 weeks of the experiment while those from plants exposed to radiations from the GSM masts exhibited varying degrees of modifications or distortions (Figs. 1 and 2). The DNA of the plants exposed to radiations from the mast either showed damages once (between 2 to 8 weeks) or twice (also between 8 to 24 weeks) as summarized in Tables I and IV. Also, a total of 91.67% damage was estimated to occur in all the samples between 2 to 24 weeks of exposure to the GSM antennae radiation. The distortions occurred in 58.33% of the samples at 2 to 8 weeks of exposure while 33.33% occurred between 8 to 24 weeks. Approximately 8.33% of the samples did not show distortions in DNA at all (sample 12 from location C 400 m). The results also showed that the DNA of plants at all the tested distances from the masts was damaged.

IV. Discussions

The abundance of distortions to DNA of *H. sabdariffa* exposed to radiation from the GSM antennae as compared with those raised in location without GSM signal showed that the radiation is harmful to DNA. This is corroborated by the report of [6] that DNase through the membranes to lysosomes lead to fragmentation of DNA seen in cells exposed to mobile phone signals and that if this occurs in germ lines, may reduce fertility and produce genetic damage in future organisms. Reference [19] also reported that UV-C-irradiated plants produce a volatile signal that triggers an increase in genomic instability in *Arabidopsis thaliana* and tomato. The level of influence the damages will have on the plants will depend on several factors which may be intrinsic or extrinsic. The intrinsic factors may include the plant health and the DNA

repair mechanisms of the plant. The extrinsic factors may be the environmental factors such as sunlight, nutrients and water availability to the plant.

The results of this study showed relatively high estimated distortions to DNA of the test plant, *H. sabdariffa*, 91.67% in all, with 58.33% of the samples between 2-8 weeks of exposure and 33.33% of the samples between 8-24 weeks exposure. Plants placed at all the tested distances to the GSM antennae showed damages to their DNA (Table I).

TABLE I
TIME DEPENDENT EFFECTS OF RAY EMISSION FROM 900 MHZ GSM MAST ON DNA OF *H. SABDARIFFA* SAMPLES

Distance (m)		Sample	Nature of Change Effect	Times of Damage
Control		Control	ABC	0
	100 m	1 ABC	A B – C	1
	200 m	2 ABC	A – B – C	2
Location A	**300 m**	3 ABC	AB - C	1
	400 m	4 ABC	A – B – C	2
	100 m	5 ABC	A – BC	1
	200 m	6 ABC	A – B – C	2
Location B	**300 m**	7 ABC	AB - C	1
	400 m	8 ABC	A - BC	1
	100 m	9 ABC	AB –C	1
	200 m	10 ABC	A – B – C	2
Location C	**300 m**	11 ABC	AC – B	1
	400 m	12 ABC	ABC	0

The relatively similar growth conditions of the soil and the weather conditions in both the control and test locations enabled the opinion that the observed differences, mainly due to radiations exposure, could be responsible for the differences observed in DNA profiles analyses (Figs. 1 and 2) of each of the exposed sample plants while those from the control remained relatively the same.

The transmitting signals of the GSM technology, although generally believed to be harmless because it has no heating effects and are non-ionizing [20], it is fast becoming an environmental hazard by its effect(s) on biological molecules, especially the DNA. In as much as the benefits of the GSM technology is becoming indispensable to our struggle to conquer the universe, it is time to channel efforts towards making it safe for living organisms who stand the risks of exposure in the ecosystem and to preserve species continuity and biodiversity.

In conclusion, this experiment showed that electromagnetic radiation from the 900MHz GSM antenna, though regarded as very low and weak is potent enough to cause distortions to DNA of *H. sabdariffa* even within 8 weeks of exposure. Radiation at all distances considered from the mast in the experiment proved to be hazardous to the DNA of the test plant. These results will help to fill information gaps in the controversies surrounding the danger or otherwise associated with radiation from GSM mast and to resolve it. It will trigger efforts to prevent further erosion of plant genetic resources which has been threatening food security and also help to protect the safety of our environment.

TABLE II
DMRT OF TEMPERATURES (˚C) AND RELATIVE HUMIDITY (%) IN THE CONTROL AND EXPERIMENTAL LOCATIONS IN RELATION TO DISTANCE FROM THE MAST

Locations	Parameter	Control	Distance 100m	200m	300m	400m
A	Temperature	31.50±3.64	31.38±3.86	31.38±3.94	31.33±3.84	31.33±3.91
	R. Humidity	58.99±15.71	58.57±16.43	58.63±16.43	58.55±16.46	58,48±16.52
B	Temperature	31.50±3.64	31.80±2.97	31.85±3.16	31.60±3.11	31.60±3.03
	R. Humidity	58.99±15.71	58.85±16.73	58.70±16.65	58.75±16.58	58.70±16.59
C	Temperature	31.50±3.64	31.11±3.65	31.50±3.58	31.55±3.85	31.45±3.88
	R. Humidity	58.99±15.71	58.90±16.60	58.88±16.54	58.80±16.48	58.93±16.58

Means in the same row are not significantly different ($p \leq 0.05$)

TABLE III
EMF INTENSITIES (V/M) OF STUDY LOCATIONS IN RELATION TO DISTANCE FROM THE 900 MHZ GSM MAST

Locations	Control	Distance (m) 100	200	300	400
A	0.02 ± 0.00^{a}	0.66 ± 0.14^{b}	0.70 ± 0.13^{b}	1.02 ± 0.31^{c}	0.94 ± 0.26^{c}
B	0.02 ± 0.00^{a}	0.40 ± 0.15^{b}	0.54 ± 0.22^{c}	0.71 ± 0.25^{d}	0.68 ± 0.23^{d}
C	0.02 ± 0.00^{a}	0.72 ± 0.19^{b}	0.82 ± 0.18^{bc}	0.99 ± 0.50^{c}	1.01 ± 0.45^{c}

Means in the same row with different superscripts are significantly different ($p \leq 0.05$).

TABLE IV
DAMAGE ESTIMATES IN *H. SABDARIFFA* DNA DUE TO EXPOSURE TO RAY EMISSION FROM 900 MHZ GSM MAST

Damage Type	Exposure Time	
	2 - 8 Weeks	8 - 24 Weeks
Single	58.33%	91.67%
Double	-	33.33%
None	-	8.33%

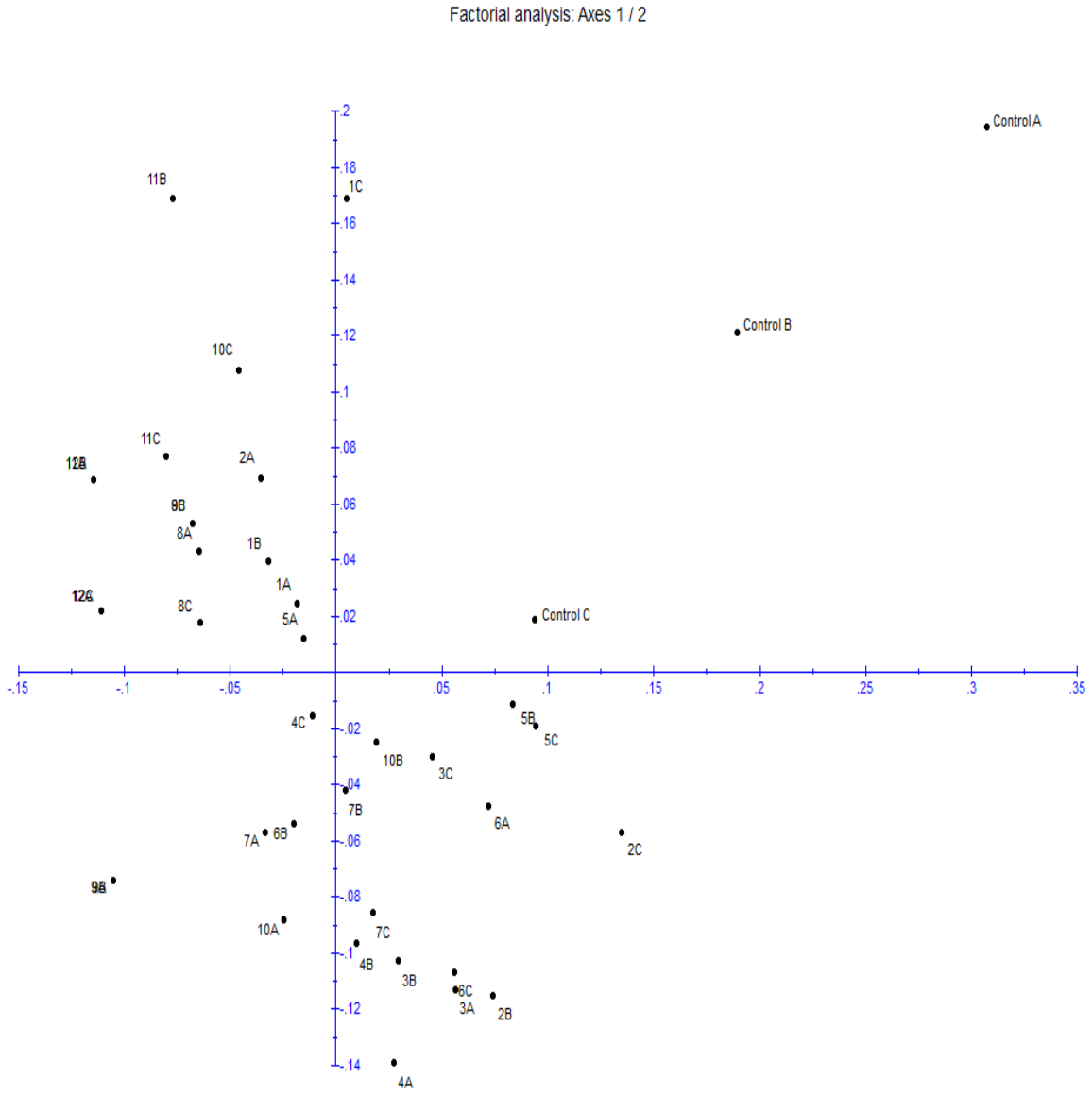

Fig. 1 Factorial analyses of DNA samples *of H. sabdariffa* from the control and those exposed to radiofrequency radiation from 900 MHz GSM antennae

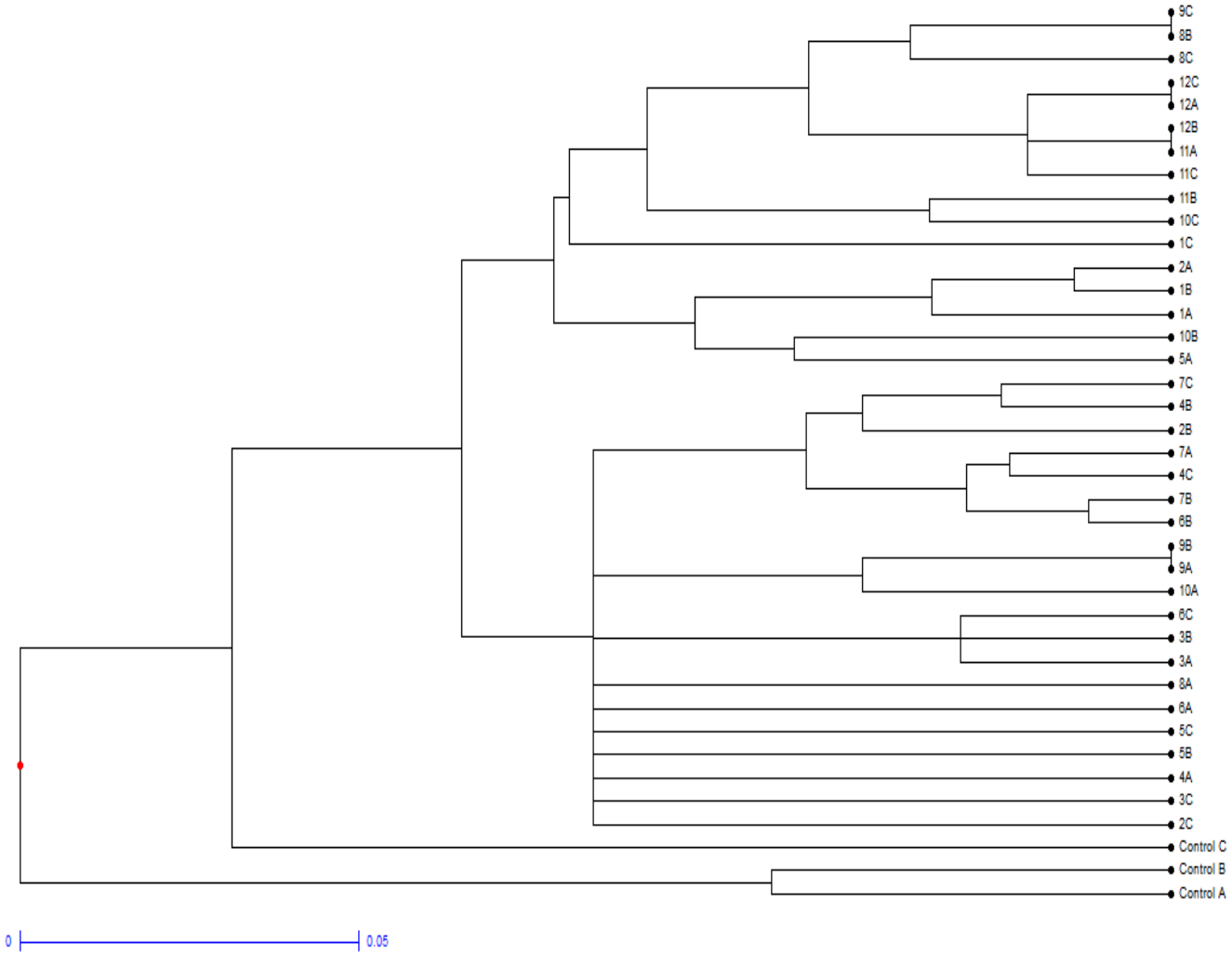

Fig. 2 Dendogram of *Hibiscus sabdariffa* samples from the control and those exposed to radiofrequency radiation from 900MHz GSM antennae

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