

Some Stomatal Aspects of *Calotropis procera* Induced by High Voltage-Power Lines

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ABSTRACT: Three different distances (0, 50 and 100m) apart from each of four high voltage-power lines which are differed in their installation date (1989, 1991, 2008 and 2013) were used to examine the effect of electromagnetic field (EMF) on stomatal opening length (SOL), stomatal density (SD) and stomatal conductance (SC) of *Calotropis procera* leaves. The magnetic flux density (MFD) generated from the recently installed high voltage-power lines (1991, 2008 and 2013) were higher than that for the old one (1981). The MFD reduced with the increasing of the distance apart from a power line.

The highest SOL was recorded just beneath the power line 2008 (16.87 μm), whereas the lower SOL (10.73 μm) was recorded at 100 m far away from the 2008-power line. The SOL for the control plants (15.17 μm) was found to be 11% less than the highest SOL and 41% more than the lowest SOL. The Stomata closer to the power line (at 0 and 50m apart) are wider than those at (100m) apart except at the 2013-power line.

The highest stomatal density (SD) was obtained at 50m-apart from 1991 power line (1240 count/mm²), while the lowest SD was found just beneath the 2013-power line (265 count/mm²). On the other hand, control plants recorded (859 count/mm²) which is 44% less than the highest value and almost double the lowest value. The wider SOL was found at the recent power lines (2008 and 2013) comparing to those at the old power lines (1981 and 1991) at the same distances.

The SC for the plants collected from beneath the power lines had higher values for the power lines except for the 2013-power line comparing to the other distances. In addition, the SC values for the control were lower than those for 1981- and 1991-power lines and higher than those for 2008- and 2013-power lines.

KEYWORDS: Electromagnetic field; high voltage-power lines; Stomatal opening length; Stomatal density.

I. INTRODUCTION

Increasing population of Saudi Arabia and fast growing economy will lead to expansion of towns and many buildings are constructed near high voltage overhead power transmission lines. Accordingly the increase of power demand has increased and the need for transmitting huge amounts of power over long distances is urgently required. Large transmission line configurations with high voltage levels (380Kv, 110Kv) and current levels generate large values of electric and magnetic fields stresses which will affect humans, animals and plants (Anonymous, 2013).

High voltage had negative effects to the growth and development of the plants and their leaf thickness and leaf mesophyll show modifications as densely packed mesophyll in leaves (Gemici et al., 2013). Plant leaves suffer damage at electric field strengths close to very high voltage power lines due to corona discharge at the tips of the leaves. Such field levels are found only at places (Matthes et al., 2000).

It was indicated that each of number of stem trichome, frequency and diameter of xylem elements as well as collenchyma cell row with thick cellulose walls was increased in electromagnetically-stressed cells compared to controls which can increase plant resistance against stress (RamezaniVishki et al., 2013). Reduced density and compactness parenchymal mesophilic cells and increasing the spaces between them along with stomatal density reduction in the stressed samples compared to control can enhance air exchange in leaves due to the increasing in

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leaf pores and pore density. Larger and more elongated vegetative meristems in treated samples compared to control may be a symptoms of more active divisions in the area affected by EMF (RamezaniVishki et al., 2013).

The effect of continuous exposure to EMF and low intensity radiations from network of cell towers on health of some plants were investigated by Geeta and Puja Kumari Singh (2014). Potential harm due to continuous exposure could be realized on plants in the manifestation of morphological such as stomatal patterning and biochemical disorders. They found that, at certain distances, stomatal density and stomatal index increased but followed sharply by a fall in values (Geeta and Puja Kumari Singh, 2014).

Stomatal density and stomatal indices increased significantly with the increasing the distance up to 150m apart from the tower center while decreased at 200m and 250m distances comparing to that for the control (Geeta and Puja Kumari Singh, 2014).

Stomatal area was reduced at the different distances apart from the tower center comparing to that for control although the maximum value was found at the 100m distance on the dorsal surface of leaves. Otherwise, higher temperatures may cause injury and death of leaf tissues (Sharma and Butler, 1975). In addition, higher temperature might have caused loss in enzymatic functions. The dimension and frequency of stomata can change more than two fold according to its moisture content or developmental stages (Jones, 1987). Stomatal indices are positively correlated with those of densities due to the differences in radiations received by the plants at increasing distances from cell tower (Geeta and Puja Kumari Singh, 2014 and Jones 1987). It was indicated that plants may be affected by radio frequency electromagnetic radiations by growth stimulation and die back (Levitt and Lai, 2010). In addition, the yield of most fruit crops reduced sharply from after few years of cell tower installation (Kumar and Kumar, 2009). The radiofrequency radiation creates electromagnetic pollution in plants as well as other living things (Havas, 2006).

Power transmission lines revealed similar stress effects of sodium chloride on endogenous hormones of indole-3-acetic acid and abscisic acid (Zhang et al., 2006).

Stomatal conductance (SC) is the measure of the rate of passage of carbon dioxide in $\text{mmol.m}^{-2}.\text{s}^{-1}$ entering, or water vapor exiting through the stomata of a leaf. Stomatal conductance is the function of the density, size and degree of opening of the stomata. It plays important role in plant water and CO_2 exchange and hence growth and development (Brungnoli et al. 1991). The rate of stomatal conductance is directly related to the boundary layer resistance of the leaf and the absolute concentration gradient of water vapor from the leaf to the surrounding atmosphere. The SC is under a direct biological control through using the guard cells, which surround the stomatal pore (Taiz and Zeiger, 1991). The CS is considered as one of the physiological processes that response to various degrees of salinity (El-Feel et al. 2013, Abbruzzese et al., 2009 and Karlidag et al., 2011). With the onset of water stress, conductance of mature leaves declined to a level near that of immature leaves. Loss of turgor in mature leaves may be a major factor in early stomatal closure (Steinberg et al., 1989).

Furthermore, salt stress has strong effect on growth, physiological responses and mineral accumulation. Salt stress resulted in reduced growth and biomass production, due to decreased stomatal conductance, specific leaf area, tannin and some minerals and increased sodium-intake (El-Feel et al. 2013). Salt stress, accompanied with shortage of water is among major stress factors under arid and semi-arid conditions that affects plant growth and survival (Pessarakli and Szabolcs, 2010)

Plants grown under high voltage power transmission lines (0.1-10 mGand 50-60 Hz) had similar responses shown to NaCl stress with different plants by regulating contents of endogenous indole-3-acetic acid and abscisic acid hormones (Gemici et al. 2013).

Exposing all the wild-type tomato plants to 900 MHz electromagnetic radiation for 10 minutes evoked rapid and substantial accumulation of basic leucine zipper transcription factor m RNA [which is known to regulate the response to salt stress (Lorenzo et al., 2009) in terminal leaf with kinetics very similar to that seen in response to wounding, while in abscisic acid (ABA) mutant, the response was more rapid, but transient (Beaubois et al., 2007).

The objective of the study was to investigate the changes in some anatomical aspects of stomata of *Calotropis procera* leaves such as stomatal opening length and stomatal density a result of exposure to electromagnetic of high voltage transmission lines.

II. MATERIALS AND METHODS

Raw Material

The wild plant, *Calotropis procera*, was used to examine effect of EMF generated from high voltages electricity transmission lines. The study was conducted at north of Jeddah province west of Saudi Arabia. Four transmission lines

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were selected according to their installation dates from the oldest to the newest one (1981, 1991, 2008 and 2013). The power of magnetic field was measured from the bottom of the tower until the power measured weak, then the effect of the magnetic field stated for three distances from the tower, namely 0 m, 50 m and 100 m. The control samples were taken from area far away from the effect of electromagnetic field. The Trifield Range Broad Band, having frequency range of 0-0.1 milliGauss was used to measure the magnetic field in each sampling site.

Sample preparation

Plant leaves were collected randomly from pre-selected experimental area. Five samples from each site were collected from each distance. Samples were numbered accordingly and submitted to lab for further analysis. The collected *Calotropis procera* leaves were thoroughly rinsed with distilled water. The samples were then dried in oven at 75° C for 48 hours until a constant weight was gained. The dried samples were then ground into fine powder and stored in fresh plastic polythene bags, ready for further use.

Characterization of leaves

Stomatal opening length (SOL) and stomatal density (SD)

Scanning Electron Microscopy (SEM) was used for imaging and measuring the SOL as well as computing the SD of *Calotropis procera* leaves. Each leaf sample was placed on a double side carbon tape on Al-stub and air-dried. After air-dehydration for the leaves tissues, all samples were sputtered before examination, with 15 mm thick gold layer (JEOL JFC- 1600 Auto Fine Coater) in a vacuum chamber (Valcineideet *al.*, 2005 and Tang *et al.*, 1997). One shrub of *Calotropis procera* was assigned at each distance apart from each of the four power lines for stomatal assessment besides the control plant. From each shrub, three leaves were chosen and considered as three replicates. Each leaf was dehydrated and gold-sputtered before investigation by SEM. The samples were examined with a SEM Quanta FEG 450, FEI, Amsterdam and Netherland at Scanning Electron microscopy Unit, Faculty of Science, King Abdullaziz University. For the picked-up images, each of SOL and SD was determined after calibration of the original ruler of each parent SEM image. The measuring and calibration processes as well as the stomata counting required for the SD computations were done using suitable vision system (OPTIKA PRO 5 Digital Camera- 4083.12).

Stomatal conductance (SC)

The SC was measured in fully swelled leaves CIRAS-3 portable photosynthetic system. Five leaves from each shrub were measured. All the measurements were done in the morning between 09:00 and 00:11 o'clock and at the abaxial site of the leaf. The apparatus was re-calibrated every measurement day as described by the manufacturer

Statistical design and analysis

The results of stomatal opening length (SOL), stomatal density (SD) and stomatal conductance (SC) were statistically examined by the analysis of variance according to the statistical design used, namely factorial experiment applied as randomized complete block design (RCBD) in two factors and three replicates. The first factor examined, namely power line's installation date had four levels based on its installation date in years, namely, 1981, 1991, 2008 and 2013 along with fifth level that represented the control one. The second factor studied was the distance apart from a tower was used in four levels, namely beneath power line (0m), 50 m-apart, 100 m apart from the tower and the control. Furthermore, each of the three replicates used in the present investigation is represented by one leaf of *Calotropis procera*.

For detecting the differences between each of SOL, SD and SC means, the analysis of variance was made for both properties as well as the least significant difference at 95% level of confidence ($LSD_{0.05}$) method to compare the differences between the means (Steel and Torrie, 1980).

III. RESULTS AND DISCUSSION

The magnetic flux density (MFD) measured at the four sampling distances (SD) containing the control one for each of the five power lines' installation date (PLID) containing the control area is presented in Table 1. It is clear that the MFD's generated from the recently installed high voltage- power lines (1991, 2008 and 2013) were higher than that for the old fashion power line (1981). Furthermore, the MFD reduced with the increasing of the distance apart from a power line (Table 1).

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Table 1: Magnetic flux density* (milliGauss) measured at the different sampling distance (SD) apart from a power line (DAPL) each of the four power line's installation date (PLID) along with the control area.

SD (m)	Power line's installation date (PLID)			
	1981	1991	2008	2013
0	6.9 ^{B_a}	8.2 ^{A_a}	8.7 ^{A_a}	8.2 ^{A_a}
50	1.9 ^{B_b}	2.5 ^{A_b}	2.1 ^{A_b}	2.4 ^{A_b}
100	1.1 ^{B_c}	1.5 ^{A_c}	0.9 ^{B_c}	0.9 ^{B_c}
Control	0			

Values with the same letter are not significant.

Capital letters to test values in the same row.

Small letters to test values in the same column.

Stomatal opening Length (SOL)

The results concerning the SOL and the SD of *Calotropis procera* leaves were presented in Figure 1. The SEM results of the SOL are presented in Figures 3-7. It can be seen from Figure 1 that the highest SOL was recorded in the plants habituated just beneath the power line 2008 (16.87 μm), followed by stomata of plants that grown at 50m apart from the power line 1991 (16.06 μm), while the lowest SOL was recorded in plants that grown at 100 m far away from the 2008-power line (10.73 μm). The control plants recorded 15.17 μm which was 11% less than the biggest opening and 41% more than the lowest value. Worth mentioning is that, stomata of all plants near a power line (0 and 50m-apart) are wider than that at 100m-apart except at the power line 2013, in which the SOL at 100m was 12.67 μm , whereas it was 11.70 μm at 50m-apart.

The reason of increasing the SOL values detected at the recently installed high voltage-power lines comparing to the old fashion lines can be attributed to their high MFD's generated from them that increases the electromagnetic stress around the plants. Plant leaves under stress can modify some of their anatomical structures by increasing stomatal area and consequently decreasing stomatal density to enhance their air exchange ability as well as other physiological functions.

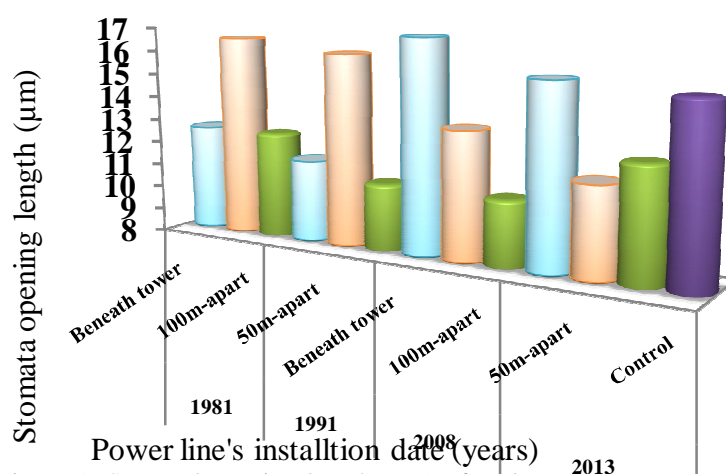


Figure 1. Stomatal opening length (μm) of *Calotropis procera* grown in the study areas in three different distances related to four different power lines and the control.

In addition, the SEM micrographs (Figures 2-6) represented SOL at the towers 1981 (Figure 2), 1991 (Figure 3), 2008 (Figure 4) and 2013 (Figure 5), at the distances of 0, 50, 100m, compared to the control (Figure 6). The results

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shows a wider SOL in the recent power lines (2008 and 2013) than those at older power lines (1981 and 1991) at the same distances, however the wider SOL was also observed in the control plant (Figure 6).

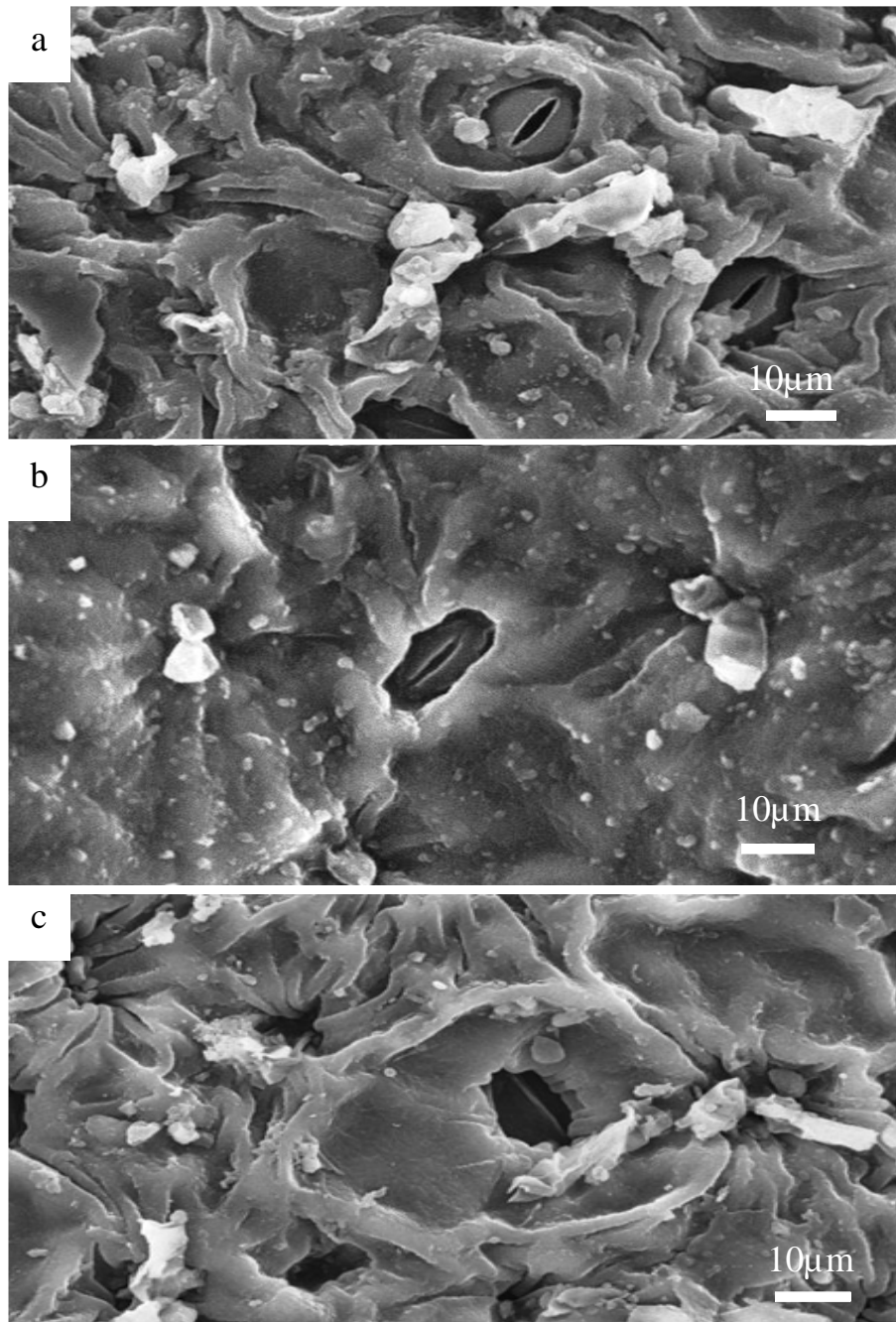


Figure 2. SEM micrographs of stomata of *Calotropis procera* leaves grown at different distances from the 1981-power line: a) beneath the power line, b) 50m apart from the power line and c) 100m apart from the power line.

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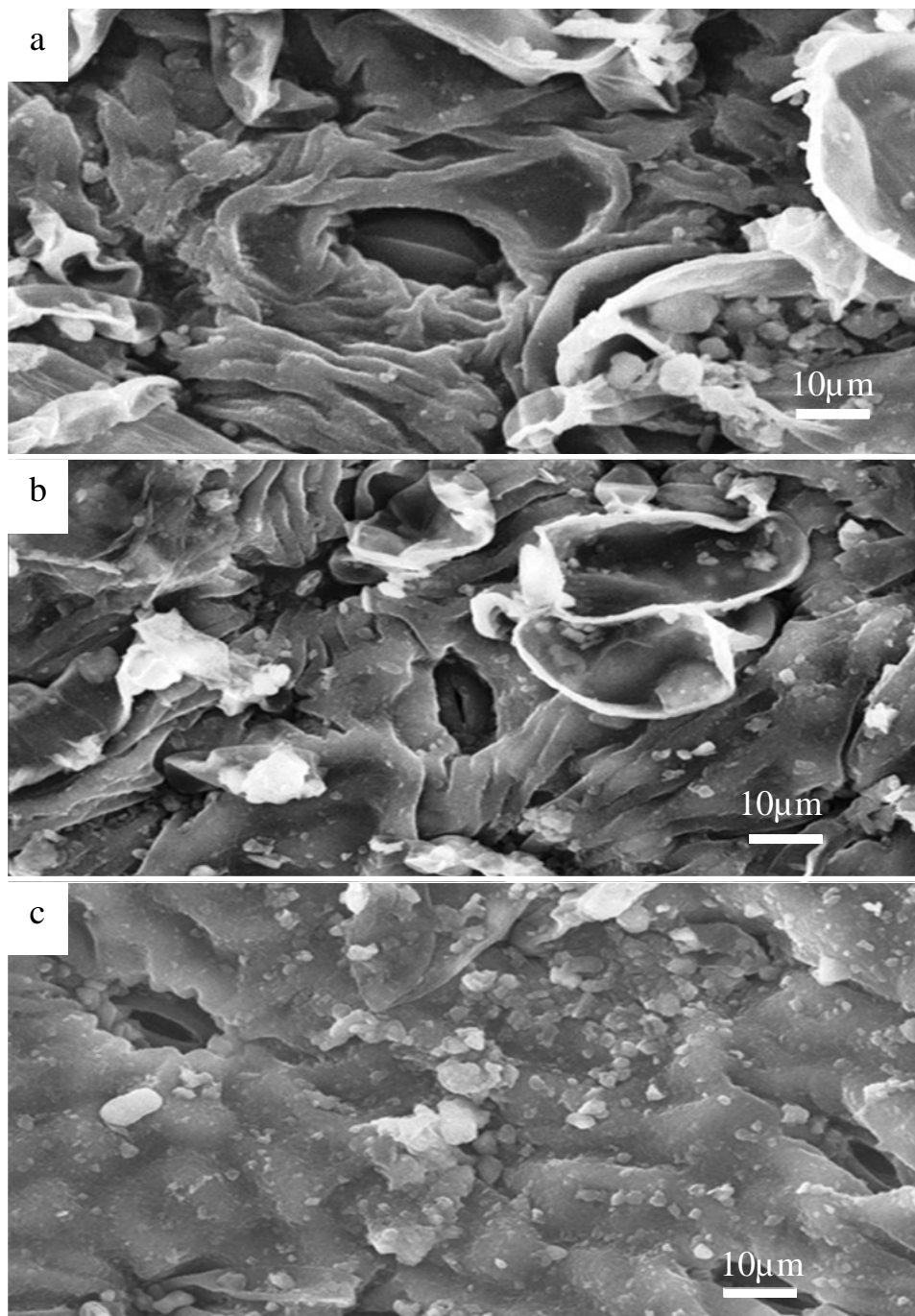


Figure 3. SEM micrographs of stomata of *Calotropis procera* leaves grown at different distances from the 1991-power line: a) beneath the power line, b) 50m apart from the power line and c) 100m apart from the power line.

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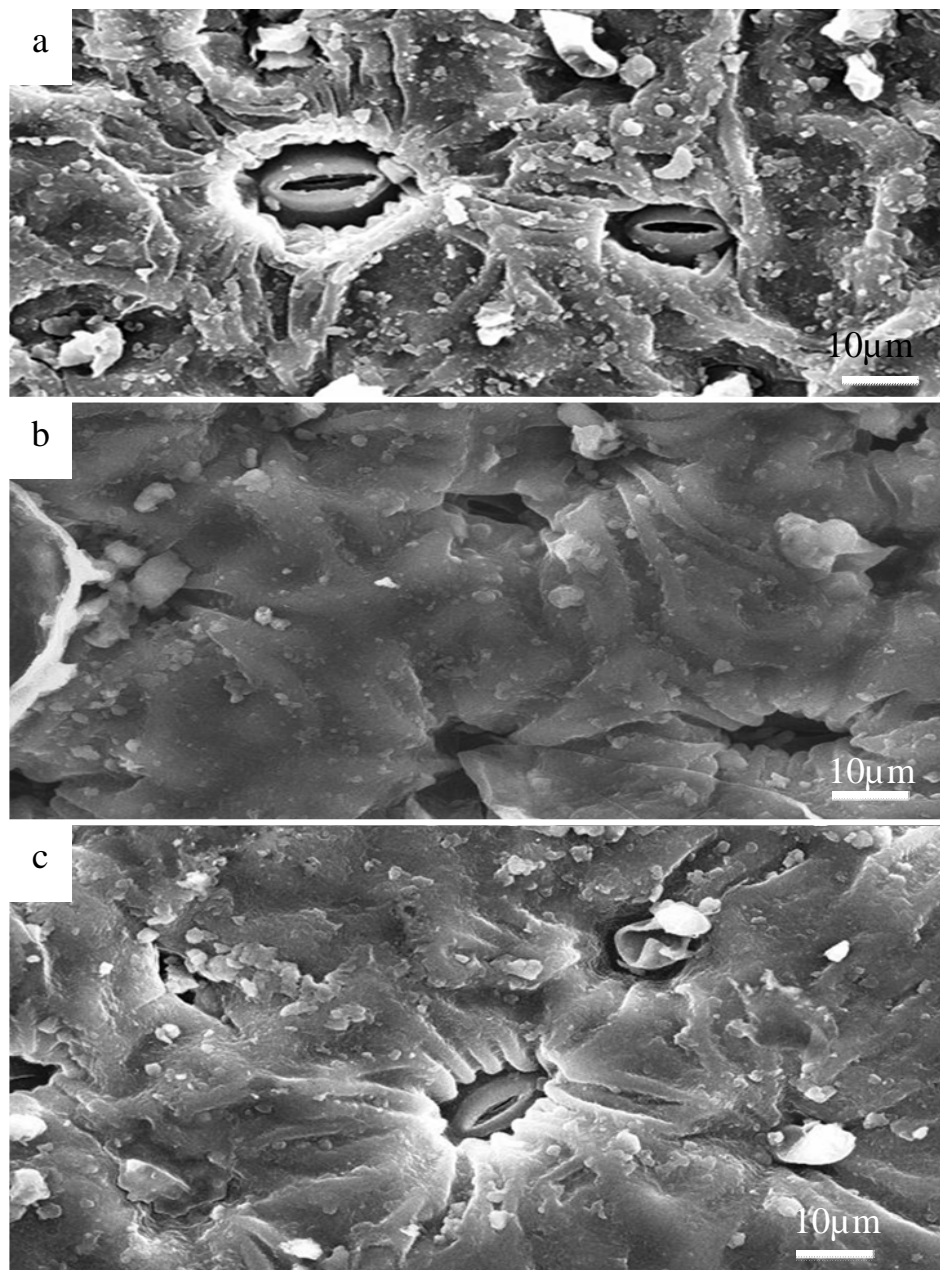


Figure 4. SEM micrographs of stomata of *Calotropis procera* leaves grown at different distances from the 2008-power line: a) beneath the power line, b) 50m apart from the power line and c) 100m apart from the power line.

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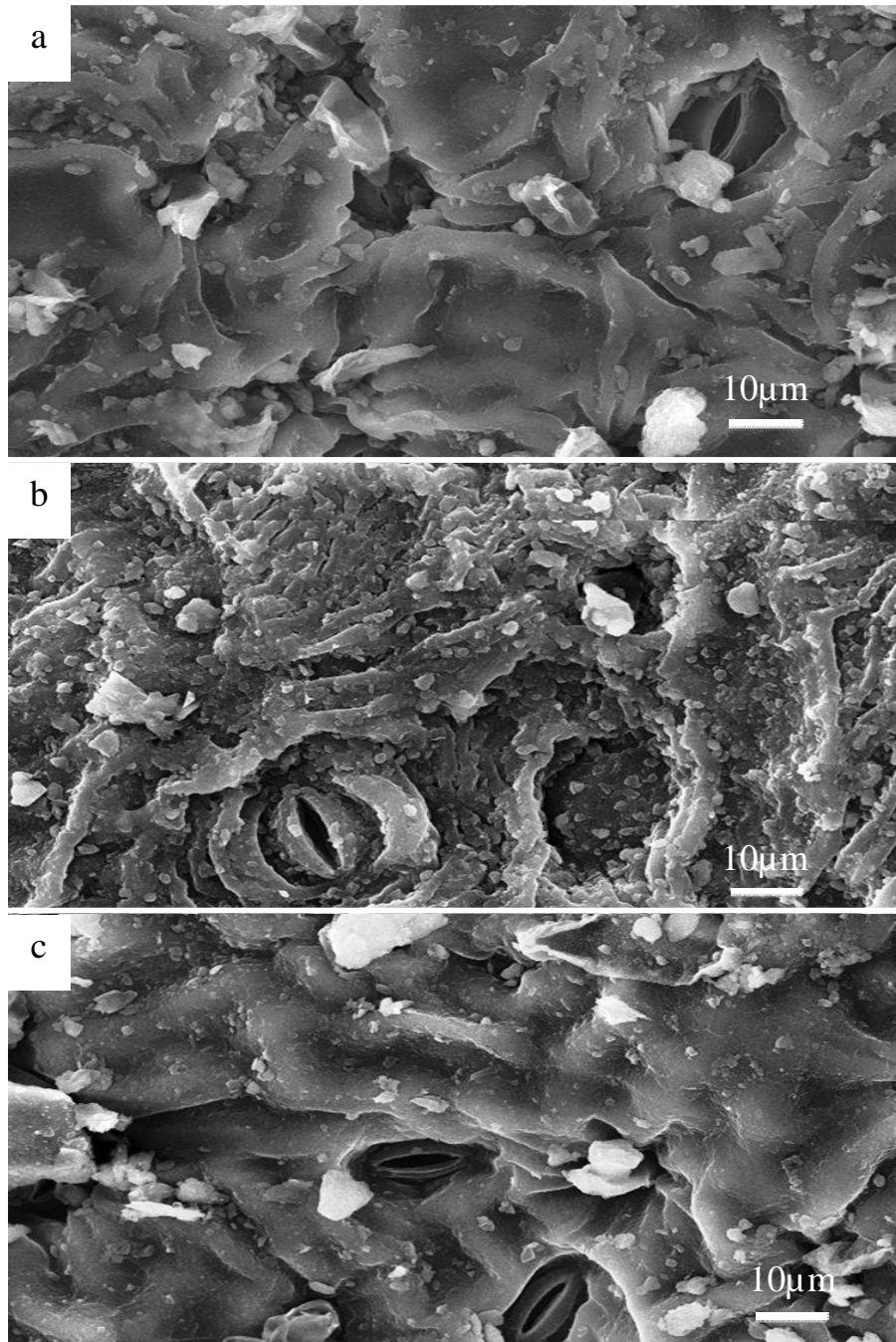


Figure 5. SEM micrographs of stomata of *Calotropis procera* leaves grown at different distances from the 2013-power line: a) beneath the power line, b) 50m apart from the power line and c) 100m apart from the power line.

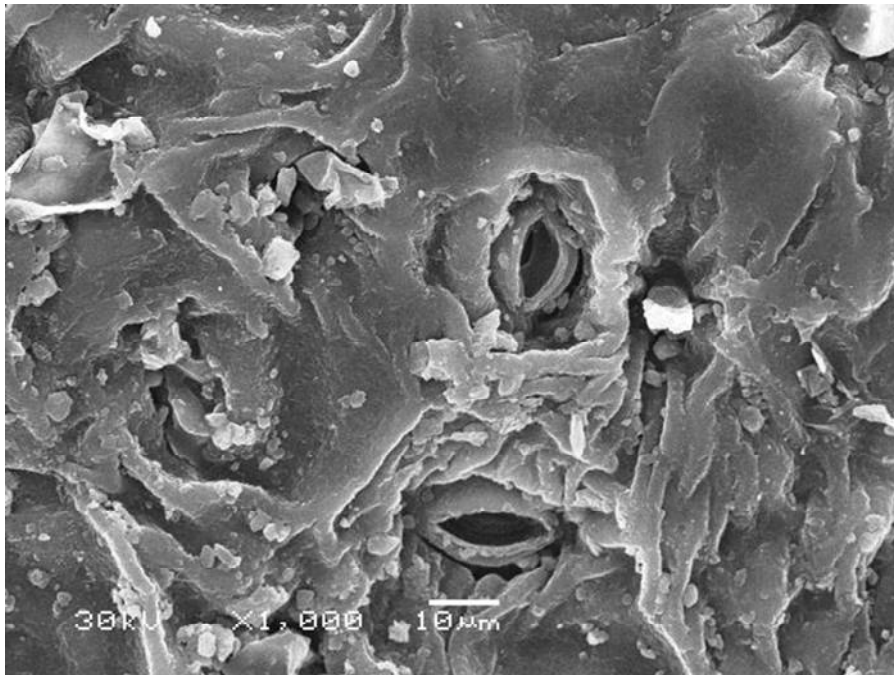


Figure 6.SEM micrographs of stomata of *Calotropis procera* leaves grown at the control area.

Stomatal Density (SD)

The results of the SD presented in Figure 7 clarified that the highest value was obtained for plants grown at 50 m apart from the 1991-power line (1240 count/mm²), while the lowest value was recorded for plants grown just beneath the 2013-power line (265 count/mm²). Alternatively, control plant recorded (859 count/mm²) which is 44% less than the highest number and almost double the less number. Moreover, plants grown at 100m apart from the 1981-power line, showed high SD (1102 count/mm²) which is 28.3% higher than the control. Furthermore, other plants recorded SD values more or less near to the values recorded for the control plant (Figure 7) except those recorded at 100m apart which showed no significant difference when compared with the control. In general, the EMF increases the DS and decreasing the stomatal area (SA) in stressed-plant leaves. In addition, the stressed leaves had wider stomata than the control.

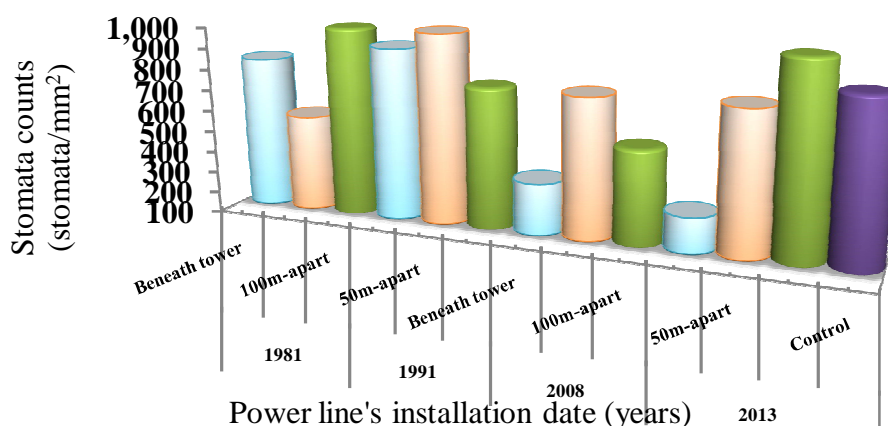


Figure 7. Stomatal density (Stomata/mm²) of *Calotropis procera* grown at three different distances related to four different power lines and the control.

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The increasing in the SD for plant leaves closer to the high voltage-power lines may be attributed to the formation of 45-amino acid peptide called “Stomagen” which is released by mesophyll cells and can induce the formation of stomata in the epidermis (Lake et al. 2001).

In addition, the SD increasing (Figure 7) may be related to the increasing of thermal effect arisen from non-ionizing electromagnetic radiations (Geeta and Puja Kumari Singh, 2014). This thermal effect is directly resulted from rubbing water molecules against each other by the oscillating electric field (Kasevich, 2000). Some researchers attributed this effect to the global warming as well as radiofrequency radiation (Chatterjee and Kar, 2014). The interpretation of high stomatal density at the closer distances to the power lines may be also attributed to the higher stomatal conductance that allows higher rate of transpiration along with gaseous exchange maintaining normal temperature within the plant body (Geeta and Puja Kumari Singh, 2014). Furthermore, higher temperatures may also reduce moisture content in plant leading to reduce photosynthesis rate and loss of turgidity in guard cells leading to closure of stomata (Geeta and Puja Kumari Singh, 2014 and Jones 1987). The dimension and frequency of stomata can change more than two fold according to its moisture content or developmental stages (Jones, 1987). Stomatal indices are positively correlated with those of densities due to the differences in radiations received by the plants at increasing distances from cell tower (Geeta and Puja Kumari Singh, 2014 and Jones 1987).

Stomatal conductance (SC)

The results of the SC for the *Calotropis procera* habituated at the investigated areas are presented in Table 2. It is clear that the SC values obtained beneath the power lines and 100 apart for the power lines installed in 1981 and 1991 were higher than those installed in 2008 and 2013. In addition, the plants grown at the power lines area installed in 2008 had the lowest values.

Generally, the SC values for the plants collected from beneath the power lines had higher SC values except for the 2013-power line than the other distances for each of the four power lines. However, the SC values of the control were lower than those for 1981- and 1991-power lines but they found to be higher than those for 2008- and 2013-poer lines.

Table 2: Mean values of stomatal conductance ($\text{mmol.m}^{-2}.\text{s}^{-1}$) measured at the different distance apart from a power line (DAPL) for each of the four power line’s installation date (TID) along with the control area.

DAPL (m)	Power line’s installation date (TID)			
	1981	1991	2008	2013
0	509 ^A _a	472 ^{AB} _a	103 ^C _b	136 ^C _b
50	285 ^B _c	341 ^A _b	70 ^C _b	320 ^A _a
100	416 ^A _b	286 ^B _c	110 ^C _b	146 ^C _b
Control	313 _c	313 _b	313 _a	313 _a

Values with the same letter are not significant.

Capital letters to test values in the same raw.

Small letters to test values in the same column.

IV. CONCLUSION

- The magnetic flux density generated from the recently installed high voltage-towers (1991, 2008 and 2013) were higher than that for the old one (1981). The MFD reduced with the increasing of the distance apart from a power line.
- The highest stomatal opening length was recorded in the plant habituated just beneath the 2008- power line, whereas the lowest one was recorded in plants that grown 100 m far away from 2008- power line.
- The control plants recorded was 11% less than the highest stomatal opening and 41% more than the small opening.
- Stomata of all plants near the power lines (0 and 50 m) are wider than that at 100 m-apart except at the 2013-power line.
- The recent power lines (2008 and 2013) gave higher stomatal opening lengths than those for old power lines (1981 and 1991) at the same distances.

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- The highest stomatal density was obtained by plants grown at 50m apart from the 1991-power line, whereas the lowest value was recorded beneath the 2013-power line.
- The stomatal density of the control plant was found to be 44% less than the highest value and almost double the lowest value.
- The plants grown 100m apart from 1981-power line showed high stomatal density which is 28.3% higher than the control.
- The stomatal conductance values obtained beneath the power lines and 100 apart for the power lines installed in 1981 and 1991 were higher than those installed in 2008 and 2013. In addition, the plants grown at the power lines area installed in 2008 had the lowest values.
- The stomatal conductance for the plants collected from beneath the power lines had higher SC values except for the 2013-power line than the other distances for each of the four power lines.
- The stomatal conductance values for the control were lower than those for 1981- and 1991-power lines and higher than those for 2008- and 2013-power lines.

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