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A preliminary electro biophysical study on electrical polarity detection ability of the excitable plant Mimosa pudica: A probable biosensor of electrical polarity

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ABSTRACT

J.C. Bose, for the first time had established that excitable plant tissue exhibits the similar excitatory response to polar electrical currents which are seen in excitable animal tissues. Recent studies have also reported electrical polarity sensing ability of the sensitive plant Mimosa. In this backdrop, the present electro biophysical study aims at understanding the characteristic response of Mimosa to polar electrical currents and the effect of variable intensities of the stimulating DC voltage on the characteristic response of the plant. For the study, a compact and customized electrical polar stimulator apparatus has been developed in the laboratory.

1. INTRODUCTION

During the early 20th century, Acharya Jagadish Chandra Bose, for the first time proved that excitable tissues of sensitive plants like Mimosa respond to polar electrical current in a manner like that of excitable animal tissues [1]. Electrophysiological experimental studies conducted in the past using nerve-muscle preparation have established that excitable animal tissues exhibit a characteristic response to polarity of electrical current [2]. In view of this, Bose, through his extensive experiments on sensitive plants like Mimosa, Biophytum and Averrhoa had demonstrated that the plants' response to polarity of electrical current is like that of animals [1]. Recent electrophysiological studies have proved that plants also employ electrical signals to modulate physiological functions [3]. Like that of nerves, in the excitable plant Mimosa pudica signals, analogous to action potentials (APs) have been noted [4, 5]. Electrical signals in Mimosa pudica are generated by various stimulations like electrical, tactile, thermal, and so on. Such stimuli induce rapid closure of leaves in sensitive plants and results in the drooping of the petiole [6, 7]. Thus, studying the peculiar responses of sensitive plants like Mimosa to polarity of electrical currents is important for better understanding of the biophysical and electrophysiological basis of information processing in plants. Moreover, recently, sensitive plants like Mimosa are considered an important model for bioengineering based experimental

KEYWORDS

Biosensor; excitatory response; mechanical response; Mimosa; polar electrical currents; variable DC-stimulus intensity

studies where identification of real direction of flow of electrons is important [8].

It was thus hypothesized that the sensitive plant may exhibit characteristic drooping in response to polarity of the stimulating DC voltage. In this backdrop, an electro biophysical study has been conducted to understand the effect of polarity of DC electrical currents on the pattern of mechanical response of the sensitive plant Mimosa pudica. For the study, customized and compact 'electrical polar stimulator apparatuses have been developed in the laboratory. This stimulator device has been employed for studying two aspects of electrostimulation – the polarity of current responsible for inducing the characteristic change and the effect of variable intensities of the polar electrical current on the mechanical response of the excitable plant, Mimosa.

1.1 Significance of the Study in context of present-day research

In the last few years several bioengineering, computational and electro biophysical based experimental and review studies have been conducted by researchers worldwide to understand the electrical and biophysical basis of information processing in the sensitive plant Mimosa pudica. A comparative tabular representation of such studies has been presented in Table 1.

Table.1ComparativeTabularRepresentationoftheresearch works on the information processing ability of the
sensitive plant Mimosa pudicaEnd

Study References	Study Highlights
Munakata et al	The authors designed a simulation
2022 [9]	model for development of a bio-
	inspired pneumatic actuator based on
	the structural properties of the primary
	pulvinus of the plant Mimosa.
Aishan et al 2022	The study was based on development
[10]	of a bio-actuated microvalve for
	microfluidic experiments based on the
	drooping and recovery response of
	petiole of Mimosa following exposure
	to external physical stimuli.
Wang et al 2021	The study focused on development of
[11]	bionic mimosa blades based on the
	mechanism of closure and opening of
	leaflets of Mimosa pudica.
Baluska and	This review work highlighted research
Yokawa 2021 [12]	works conducted in the past and present
	times concerning plant neuroscience,
	presence of a sensory pathway and
	cognitive behavior in plants.
Mano and Hasebe	This review work summarized the
2021 [13]	present understanding of faster and
	short duration movements of excitable
	plants.
Awan et al 2018	The authors characterized the
[14]	information-theoretic aspects of
	different communication signals in
D 1 2014	plants.
Basir et al 2014	The study was based on designing and
and 2015 [15]	analysis of a bio-mimicked touch
	sensitive sensor based on the functional
	mechanism of the pulvini, the motor
T 1	organ of <i>Mimosa</i> .
Jovanov and	The authors discussed the different
VOIKOV 2012 [16]	methods of applying electrical
	sumulation in excitable plants. They
	also designed a novel DC based method
	or electrical stimulus application in the

2. MATERIALS AND METHODOLOGY

2.1 Plant Procurement and Maintenance

For the present study a full-grown Mimosa pudica plant was procured from a nursery and acclimatized in the laboratory for a period of seven days. For acclimatization, a specialized plant acclimatization chamber (Figure 1) was developed in the laboratory where the crucial physiological parameters for plant growth could be well-regulated.



Fig. 1 Acclimatization of Mimosa pudica inside the labbuilt Plant Acclimatization Chamber

2.2 Development of the lab-built Electrical Polar Stimulator Apparatus

2.2.1 The Electrical Unit (Figure 2): The electrical unit (K) consists of a DC-DC step up module (XL6009) and a 3.7 V Lithium ion (Li-ion) battery with charging module. The battery acted as the DC source of voltage. The step up module was connected to a 100k potentiometer to regulate the voltage intensity. The range of the DC voltage was 3.78V - 57.0 V. A DPDT switch was integrated into the electrical unit in order to swiftly alter the polarity of the electrical current at the time of experimentation. The unit also contained a digital voltmeter that enabled monitoring of the voltage intensity corresponding to the characteristic plant response.

2.2.2 The Mechanical Unit (Figure 2): The mechanical unit D consisted of a pair of electrodes holding stands. These stands in turn were made up of two arms – vertical arm A and horizontal arm B. The position of both of these arms could be varied lengthwise by the help of the adjusting screw C. The horizontal could arm rotate horizontally with respect to axis (Q) and vertically with respect to axis (R). The tip of the horizontal arm was provided with an electrode holder F. The electrode holding stands were connected perpendicularly to a base. The output connections of the electrodes were in turn coupled to the H1 and H2 banana jacks of the electrical unit K.



Fig. 2 A - Schematic Diagram and B – Photographic Image of the lab-built 'Electrical Polar Stimulator Apparatus'; E1 and E2 – 2 Copper Electrodes, M1 and M2 – Pulvinus 1 and 2, L1 and L2 – Leaflet 1 and 2, S – Stem, P – Plant, K – Electrical unit, D – Mechanical unit

2.3 Electrode preparation

The two stimulating electrodes were made up of fine copper wires (diameter 0.08 mm approximately). One end of the electrode was connected to the electrode holder F and the other end was connected to the pulvinus of the plant. The electrodes were coiled (coil diameter around 5 mm) into a spring-like structure that allowed a free variation of the length (Figure 2). This helped to overcome any sort of mechanical obstruction at the time of drooping of the petioles of the plant when connected to the electrodes. The electrodes were connected to the surface of the pulvinus by means of a Kaolin paste, that served as the impedance matching solution. The Kaolin solution comprised of 2 ml of 0.9M NaCl solution, 2.2 g of Kaolin powder and 1ml of glycerin solution [1]. The impedance matching solution was coated at the contact point of electrode and pulvinus surface through a fine brush.

2.3.1 Circuit Diagram of the Electrical Unit: Figure 3 represents the internal circuit diagram of the electrical unit of the stimulator apparatus. The Li-ion battery (B) was connected to the charging module (W). The output of the charging module was connected to input of the DC – DC step up module D through a power switch S1. The step-up module D was integrated with a 100k potentiometer (VR). A voltmeter (V) was connected to the output of the step-up module. Finally, the output of the step-up module was connected to the step-up module was connected to the output of the step-up module is power switch S2. The standard internal circuit details of the D segment of the electrical unit have been represented in Figure 4 as obtained from XL6009 datasheet [17].



Fig. 3 Internal Circuit Diagram of the Electrical Unit K of the Stimulator Apparatus



Fig. 4 Basic Circuit Diagram of the Segment D of the Electrical Unit (DC-DC Step up)

2.4 Experimental Procedure

Before starting the experiment, the surface electrodes were connected to the target petioles by means of the mechanical unit of the stimulator apparatus. Following this, the plants were allowed to recover to their normal state for about 30 minutes, as confirmed by the upright petiole position. After connecting the surface electrodes to the surface of the petioles, the output connection of the electrodes was coupled to the H1 and H2 jacks of the electrical unit. The electrical unit of the stimulator apparatus was first switched on using the power switch S1. The DPDT switch S2 was initially maintained at off condition. The desired voltage intensity was regulated through potentiometer and monitored using the voltmeter. The polarity of the two electrodes was altered using the DPDT switch. When the left petiole (L_p) was made cathodic (negative polarity) the right petiole (R_p) automatically turned into anode (positive polarity). Similarly, when the R_p was made the new cathode by operating the DPDT switch S2, the L_p became the anode. Under both the conditions, i.e., L_p cathodic and R_p anodic and vice versa the characteristic response of the petiole of the sensitive plant Mimosa was noted.

The petiole drooping response was quantified in terms of the difference in Y-axis pixel value between initial position of the tip of the target petiole and final position of the petiole tip (after drooping) within 0 to 5 s of the stimulus application. The pixel values were obtained using Paint software from the screenshots of the videographic recordings during experimentation. For obtaining a constant experimental frame a stable recording set up was assembled by maintaining a consistent angular distance between the camera lens and the subject (plant). For the ease of calculation the pixel values were later converted to mm scale.

At the time of experimentation, the ambient temperature and luminance was measured using a digital thermometer and a lux meter. The hardware specification of the mechanical and electrical unit of the stimulator apparatus has been presented in Table 2. Following this, the effect of variable voltage intensities on the polar response of the plant was also studied. Before starting the experiment, a video-camera setup was mounted in front of the whole experimental set up to keep record of the events throughout the experiment. Following drooping of the petioles on application of the stimulating voltage, a recovery period of 30 min was provided to allow the petioles to return to its normal position.

The waveform of the variable intensities of the applied (for about 7 s) single DC pulse as obtained from the oscilloscope has been represented in Figure 5.



Fig. 5 Nature of the variable intensities of the applied single DC pulse as obtained from oscilloscope (TBS 2000 Series, Tektronix)



Fig. 6 Graphical Representation of the effect of variable intensities of the voltage (DC) on the mechanical drooping response of the petiole of the Cathodal Side

 Table. 2 Hardware Specification of the Electrical Polar

 Stimulator Apparatus

Components of	Specifications
Electrical Unit	Specifications
DC-DC Step up	> Fixed 400KHz switching frequency
Module (XL6009)	> Input Voltage range: - 0.3 to +36 V
	> Output switch pin voltage: -0.3 to +60 V
	> Maximum Input current: 4 A
Li-ion Battery	> 3.7 V, 1800 mAh
DDDT Switch	> 3 A
Digital Voltmator	> 0.29 inch display
Digital volumeter	> Measuring range 0 to 100 V (DC)
Digital Multi	> 50 to ± 300 degree
Thermometer (ST	> -50 to + 500 degree
9269)	
Components of	Specifications
Mechanical Unit	
Vertical and	> Length $-$ 23 cm, Diameter $-$ 3.8
Horizontal Arms	mm
Insulated Electrode	> 32 Gauge diameter
Wire	

 Table. 3 Effect of Variable Intensities of DC Voltage on

 Mechanical Response of Mimosa

Stimulating Voltage	Y-axis Displacement (mm) following Petiole Drooping during 5 s		
Intensity (V)	Anode	Cathode	
9.10	12.7 (Strong response)	9.26 (Strong response)	
5.25	15.08 (Strong response)	8.47 (Strong response)	
4.58	0 (No response)	7.14 (Strong response)	
3.81	0 (No response)	1.85 (Feeble response)	

Table. 4 The Characteristic Effect of Anode and Cathode on Mechanical Response of Petiole at Stimulating Voltage Intensity of 4.58 V

Petiole	Y-axis Displacement (mm) following Petiole Drooping during 5 s		
	Anode	Cathode	
Left Petiole (Lp)	0 (No response)	12.7 (Strong Response)	
Right Petiole (Rp)	0 (No response)	11.11(Strong Response)	

3. RESULTS

In the present study 4 different intensities of DC voltage

were used – 9.10 V, 5.25 V, 4.58 V and 3.81 V. The results of the present study indicated that when a strong intensity of voltage was applied, i.e., 9.10 V both anode and cathode induced petiole drooping. Following this when the voltage intensity was reduced to 5.25 V a similar inducing effect of both anode and cathode was observed. However, when the intensity was reduced to 4.58 V only cathodal electrode induced petiole drooping and the anodal side did not show any drooping response. Based on this, when the voltage intensity was further reduced to 3.81 V cathode induced only a feeble drooping response, but anode did not induce any drooping of petiole.

In the present study the net Y-axis displacement following petiole drooping during 5 s under different intensities of DC voltage was quantified and it was found that under voltage intensities of 9.10 V and 5.25 V the displacement was 12.7 mm (anodal), 9.26 mm (cathodal) and 15.08 mm (anodal), 8.47 mm (cathodal), respectively. In the case of voltage intensities of 4.58 V and 3.81 V the displacement was 0 (anodal), 7.14 mm (cathodal) and 0 (anodal), 1.85 mm (cathodal), respectively. From the study it was found that in case of the anodic petiole only under high voltage intensities i.e., 9.10 V and 5.25 V there a prominent petiole displacement however, under low voltage intensities i.e., at 3.81 V and 4.58 V there was no displacement of the petiole after application of the stimulus. In contrast, in the case of the cathodic petiole there was a prominent petiole displacement noted under each of the applied DC voltages. Consequently, the effect of variable intensities of the applied voltage on mechanical drooping response of the cathodic petiole has only been considered for graphical representation (Figure 6).

Following this the effect of 4.58 V was further studied to confirm the characteristic drooping response of the anode and cathode. It was found that at voltage intensity 4.58 V the cathode induced a strong drooping of petiole, but the anode did not induce any petiole drooping. The characteristic petiole drooping response was maintained even when the polarities of the left and right petiole were altered. The effect of cathode and anode on response of petiole under the different voltage intensities have been represented in Table 3 and Table 4. At the time of experimentation, the ambient temperature was maintained at 25.3 °C and the ambient luminance was maintained at 3092 Lux.

4. **DISCUSSION**

Electrophysiological studies on excitable animal tissues (nerve-muscle preparation) conducted in the past have established the fact that when the proximal end of the nerve is connected to negatively charged electrode i.e., cathode, the indicating muscle shows contractile response [1]. Bose in his pioneering experiments on excitatory polar effects of currents on excitable tissues of *Mimosa* have established that under bipolar method of excitation, i.e., when the electrodes are connected to the pulvini of two petioles, the cathodic petiole showed drooping response but there was no such response at the anodic side [1]. In the year 1970, a study focused on development of stimulating apparatus of radio-frequency range to stimulate the pulvini differentially, depending on the polarity of the charge [11]. An experimental study in the recent past has also reported that the *M. Pudica* is sensitive to the negative pole of a DC source and can easily recognize the positive and negative pole of a battery [18].

In the present study, at a particular voltage intensity of 4.58 V coinciding characteristic response has been found (Table 4). It was noted that the cathodic petiole showed a strong drooping response, and the anodic side did not show any drooping response. Each set of experiments was repeated thrice and each time the results obtained were the same. To confirm the role of cathode in inducing the excitatory drooping of petiole, the polarity of the two petioles i.e., L_p and R_p were altered using the DPDT switch S2 and under both the states the effect of anode and cathode were studied simultaneously. As evident from Figure 7 and 8 and Table 3 when the left petiole L_p was made cathodic a strong drooping response occurred, but the right petiole R_p which turned anode did not show any drooping response. Similarly, when the right petiole R_p was made the new cathode, it also showed a strong drooping response and the left petiole which turned anode did not show any sort of drooping response.



Fig. 7 A - Before Stimulation, B - After stimulation Left petiole (electrode E1) cathode, Right petiole (electrode E2) anode.



Fig. 8 A – Before Stimulation, B – After stimulation Left petiole (electrode E1) anode, Right petiole (electrode E2) cathode.

In the study both the electrodes, i.e. anode and cathode were connected at the motile or responding organ of the plant i.e.. the pulvinus. The pulvinus is the motor organ of *Mimosa* which shows elastic properties. Studies have found that electrically or mechanically induced movements of the petiole are accompanied by a change of the pulvinus shape [19]. Since the excitable plant *Mimosa* exhibits the ability to recognize the negative and positive pole of a battery and responds accordingly at voltage intensity, this model excitable plant may serve as a biosensor of electrical polarity. A bioengineering based study conducted in recent times has also highlighted the polarity sensing ability of *Mimosa*. The study also focused on developing an equivalent electronic model for considering *Mimosa* as a natural bio-electrical polarity sensor [8].

In the study, another striking effect of the polar electrical currents on mechanical response of *Mimosa* was noted (Table 3). It was found that under stronger voltage intensities the characteristic effect of anode and cathode on drooping of petiole undergoes a marked variation. In the case of strong intensity of applied voltage, i.e., at 9.10 V and 5.25 V both electrodes, anode and cathode resulted in excitatory drooping of the petiole unlike that of stimulus intensity 4.58 V where only the cathode induced the excitatory drooping. Such variations in the characteristic response of *Mimosa* under variable intensities of polar electrical current may be used to design several bio-inspired sensors [20, 21, 16].

5. CONCLUSION

From the present study it may be concluded that the sensitive plant *Mimosa* exhibits the capability to sense the polarity of the DC form of electrical current. The present study for the first time reported the characteristic response curve of the excitable plant following exposure to variable

intensities of the DC polar electrical current. The present study indicated that at certain voltage intensity (4.58 V) the cathode i.e., the negative electrode was responsible for inducing the characteristic and excitatory drooping of the petioles. However, when the voltage intensity was increased i.e., at 5.25 V and 9.10 V both the electrodes, anode and cathode were responsible for inducing the drooping of the petioles. Soon, understanding the electro biophysical basis of such a novel characteristic response pattern of *Mimosa* under different intensities of polar electrical current may be effective in developing a wide range of biological sensors.

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